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CONTENTS

ILLUSTRATIONS

Albert Cornelius Crawford

Albert C. Crawford was born in Baltimore on June 10, 1869, and died in San Francisco, where he was Professor of Pharmacology in the Leland Stanford Junior University Medical School, March 14, 1921. He was a student at The Johns Hopkins University during the years 1888 to 1890 and again from 1891 to 1894. He received the Degree of Doctor of Medicine from the College of Physicians and Surgeons of Baltimore in 1893. From 1894 to 1900 he was Assistant in Pharmacology in The Johns Hopkins University. From 1904 to 1919 he was first pharmacologist to the Bureau of Plant Industry and later to the Bureau of Animal Industry of the United States Department of Agriculture. In 1910 he was elected to the Professorship of Pharmacology at Leland Stanford Junior University, which position he held until the time of his death. Dr. Crawford was an indefatigable student and investigator. His earliest papers deal with purely medical subjects, but after becoming an Assistant in Pharmacology he devoted all his efforts to researches in that field. The subject of the physiological standardization of drugs occupied much of his thought for many years and his papers in this field give evidence of a most thorough knowledge of the literature of the subject. His review of the chemical work done on the active principle of ergot, published in 1911, is a good instance of his thorough treatment of questions of this nature. Possessing a knowledge of many languages and being a voracious reader, no man in America had a wider knowledge of the literature of pharmacology and its allied branches than had Crawford. His papers are notable for the thoroughness with which he searched all publications that had a bearing on the subject in hand. He made valuable contributions to our knowledge of poisonous plants, such as Johnson grass, the larkspur, white snakeroot, mountain laurel, and mistletoe. From American mistletoe he isolated p-hydroxy-phenylethylamine and proved that this substance is responsible for the pressor action of mistletoe extracts. Loco weed, which in some western states was at one time the cause of great losses among live-stock, received careful study at his hands. Of late years he published researches on the chemical properties of diphtheria antitoxins, the pressor compound of the pituitary gland and certain reactions of testicular principles.

Crawford was a successful and enthusiastic teacher, a tireless investigator, a man of wide learning in his field and filled with an enthusiasm for science. His friends had every reason to hope for many more years of continued and fruitful research for him, and his loss at the height of his activity will be deeply felt.

<div align="right">J. J. A.</div>

ON THE RELATIVE AMOUNTS OF DEPRESSOR AND BRONCHO-CONSTRICTOR SUBSTANCE OBTAINABLE FROM THE ANTERIOR AND POSTERIOR LOBES OF THE FRESH PITUITARY GLAND

REV. JOHN ROCA, O.D.C.

From the Pharmacological Laboratory of the Johns Hopkins University

Schäfer (1) and his pupils furnished the first positive evidence that both a pressor and depressor principle can be extracted from the excised pituitary body. No one, so far as I am aware, has however heretofore attempted to estimate the relative amounts of depressor material in the two main divisions of the pituitary body—the anterior and posterior lobes. In fact, it has only recently become possible to separate the blood pressure raising and oxytocic substance from the blood pressure lowering substances of the organ under consideration. Abel and Nagayama (2) have found that the addition of mercuric chloride or mercuric sulphate to a rather concentrated pituitary extract, as say a "20 per cent extract," precipitates the pressor and oxytocic hormone while the depressor substances remain in the filtrate. These authors have also found that half an hour's boiling of a pituitary extract with 1 per cent HCl abolishes every trace of pressor activity and causes, indeed, a complete reversal of the blood pressure action—in place of a marked rise of the arterial pressure we now have a decided fall of pressure. This observation then gives us a ready method of comparing the blood pressure lowering, broncho-constrictor and other physiological properties of the two lobes of the pituitary organ. One has only to boil the extracts of the two lobes for the same length of time with equal quantities of acid to obtain material for the comparisons here outlined.

1

A preliminary experiment in which only the depressor and broncho-constrictor actions of the two lobes were studied may here be given.   Ox glands, 60 in number, were collected by myself and an attendant at a local slaughter house.   Immediately after the skulls were opened (from ten to thirty minutes after the animals had been killed)the glands were removed and handed to us.   The posterior lobe together with that part of the *pars intermedia* which adheres to it was separated from the anterior lobe.   As is well known, much the smaller part of the *pars intermedia* remains attached to the anterior lobe.   As fast as the separation was made the two lobes were cut into small pieces which were dropped into an appropriate volume of a boiling 0.2 per cent solution of acetic acid.   According to Houssay (3) the anterior lobe of the pituitary of the ox weighs 0.4 gram on the average while the posterior lobe weighs 2 grams.   The few weighings which have been made by us support this statement.   The volumes of dilute acetic acid into which the divided and separated lobes were thrown were therefore made up in the proportion of 1 for the posterior to 5 for the anterior lobes.   Before leaving the slaughter house the contents of the two flasks were boiled once more for five minutes, the mouths of the flasks being closed with cotton plugs.   In the laboratory the liquid of each flask was decanted off and the solid portion ground up as thoroughly as possible with washed sand and well washed with 0.2 per cent acetic acid at the pump.   The two liquids were now concentrated on the water bath under the electric fan until the posterior lobe extract amounted to about 30 cc. and the anterior lobe extract to 150 cc.   Enough 25 per cent hydrochloric acid was now added to each of the solutions to give it a content of 1 per cent of hydrochloric acid and each was boiled for half an hour at the back flow condenser.   As stated above this procedure entirely abolishes the pressor action of a pituitary extract.   After cooling a little flocculent matter that had made its appearance was removed by filtration.   The two solutions were then nearly neutralized, the reaction being allowed to remain slightly on the acid side of litmus, sterilized by boiling and placed on ice over night.   The following morning the two fluids were made up to a volume of

30 cc. and 150 cc. respectively.[1] Figure 1 shows how greatly equal weights of the fresh anterior and posterior lobes differ in their content of depressor material. One cc. of posterior lobe extract is certainly about seven times more powerful as a blood pressure lowering agent than 1 cc. of the corresponding anterior lobe extract. Figures 2 and 3 show how much more powerfully
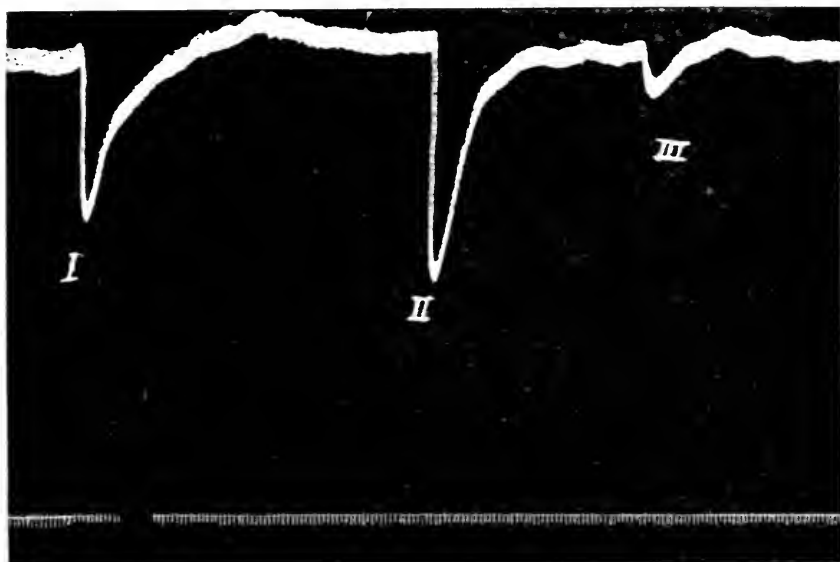
FIG. 1. DOG, MALE, 6 KGM., ETHER ANESTHESIA

Comparative action of aqueous extracts of the anterior and posterior lobes. At (I) injected into the femoral vein 5 cc. of aqueous extract of the anterior lobe. At (II) injected similarly 1 cc. of the corresponding posterior lobe extract. At (III) injected similarly 1 cc. of anterior lobe extract, same as used in (I).

aqueous extracts of the posterior lobe act in the way of constricting the bronchi than do corresponding extracts of the anterior lobe. The method of registering the constriction of the

[1] Unfortunately the notes in regard to these volumes have been misplaced and we cannot be certain that the precise volumes here given are the correct ones. It was planned to bring the solutions finally to these volumes and the figures as given are very near the true ones in any case. The ratio of the two extracts, namely 1 volume of the extract of the posterior lobe to 5 volumes of the extract of the anterior, is however absolutely correct.

bronchioles, here employed, is that of Jackson (4). In figure 4 a further illustration of the broncho-constrictor action of a posterior lobe extract is given. It may be stated at this point
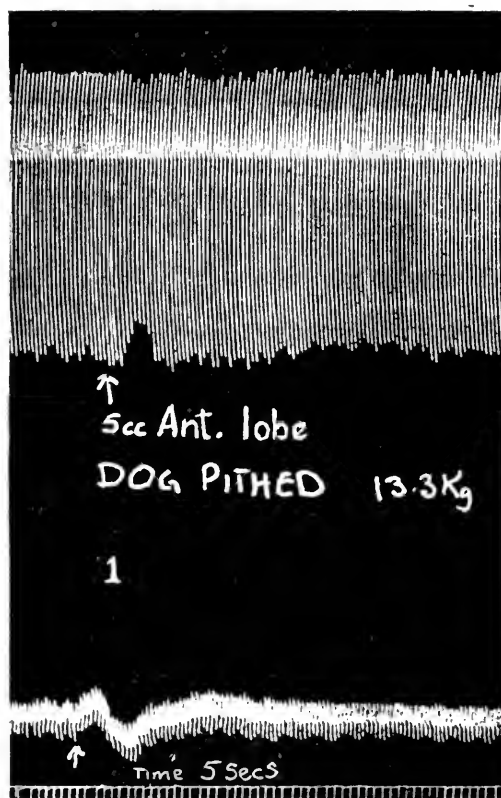
FIG. 2. DOG PITHED, 13.3 KGM.

Registration of bronchial constriction by Jackson's method. At (I) 5 cc. of the aqueous extract of the anterior lobe. Time interval: 5 seconds. Injections were made into the femoral vein. Upper tracing lung volume. Lower tracing blood pressure.

that very decided bronchial constriction may also be induced by means of anterior lobe extracts, but very large doses must be employed to obtain such an effect.

COMPARATIVE ACTION OF CHLOROFORM EXTRACTS OF THE ANTERIOR AND POSTERIOR LOBES

Abel and Nagayama (2) have adduced evidence which in their opinion goes to show that the true hormone of the posterior lobe is a single substance which has both pressor and plain muscle
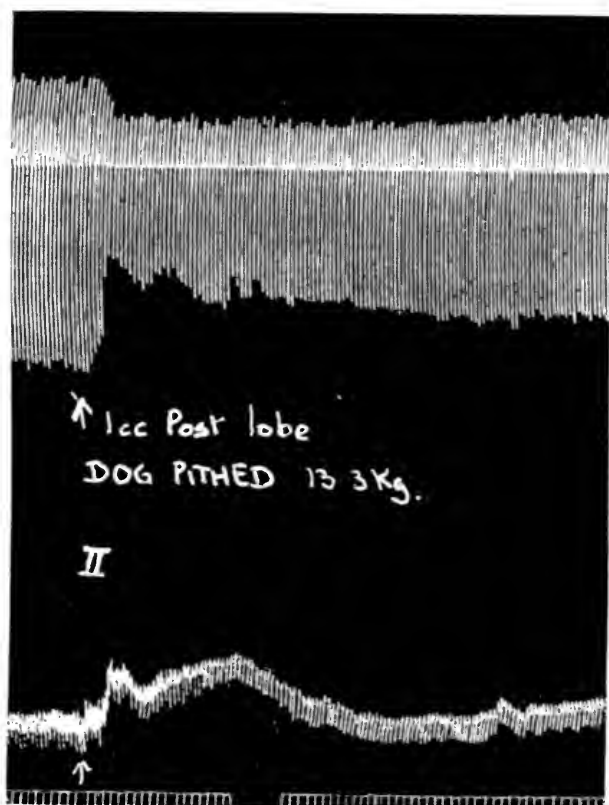
FIG. 3. DOG PITHED, 13.3 KGM.

Registration of bronchial constriction by Jackson's method. At (II) 1 cc. of the aqueous extract of the posterior lobe. Time interval: 5 seconds. Injections were made into the femoral vein. (The rise of arterial pressure at (II) does not indicate that the pressor principle is still intact. Hydrolysed extracts of the posterior lobe which never under any circumstances cause anything but a fall of arterial pressure in anesthetized, unpithed dogs, quite frequently induce a much more pronounced rise in arterial pressure than was seen in the above tracing.)

stimulating properties. This substance they have for the moment designated as " A." It is not difficult to separate this substance, although not in a pure state, from the other constituents
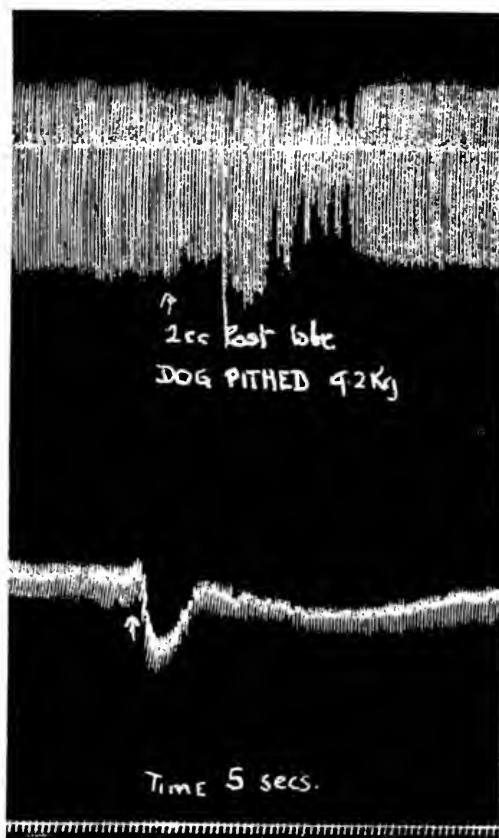
FIG. 4. DOG PITHED, 9.2 KGM.

Registration of bronchial constriction by Jackson's method. Effect of the injection of 2 cc. aqueous extract of the posterior lobe into the femoral vein. Different animal from that used in figure 2.

of the gland. A second constituent which, as far as it has been studied, exhibits histamine-like properties was called "B" by Abel and Nagayama. It must remain uncertain for the present whether or not "B," which like "A" is insoluble in

chloroform but soluble in alcohol, exceeds histamine in physiological activity, as also whether or not it agrees in all of its actions with histamine. As stated by Abel and Nagayama (2) this substance "B" (or aggregation of substances) has not yet been examined from all these points of view. A third constituent of the pituitary organ is histamine as Abel and Kubota (6) have shown and this constituent they have named "C."

Hanke and Koessler (7) have failed to detect histamine in the fresh pituitary gland and have intimated that the histamine which was isolated by Abel and Kubota, and which Hanke and Koessler admit to be histamine, must have been of bacterial origin. This histamine was separated by Abel and Kubota from the constituents of the gland by means of chloroform. One purpose of my research quite aside from a comparison of the activities of the posterior and anterior lobes was to prepare chloroform extracts from glands collected by myself and immediately sterilized by means of mercuric chloride. If chloroform extracts made with these precautions should possess all of the properties of the extracts studied by Abel and his colleagues, there would be little doubt of the correctness of Abel's position with respect to histamine. It will be seen that my results give ample support to his views, even though I have not isolated the histamine which I have every reason to believe is one of the constituents of the highly active chloroform extracts here described. Others, however, working with larger amounts of material, will later attempt to furnish conclusive proof of the presence of histamine in fresh glands.

DETAILS OF THE METHODS USED

Ox glands (430 in number) were collected by me personally, as in the first experiment, at a local slaughter house. In this instance, however, the separated lobes, after having been cut into small pieces, were dropped into two separate flasks each of which contained a 2 per cent solution of mercuric chloride [in 0.9 per cent HCl] instead of into a hot 0.2 per cent solution of acetic acid as in the first experiments. I shall here give the details of my method as applied to the posterior lobes only, merely stating that the anterior lobes were treated in the same manner.

except that larger amounts of solvents and reagents were employed, the ratio of the volumes of solvent being as 5 for the anterior to 1 for the posterior lobes.

After the material had been brought to the laboratory, the liquid was decanted off and the solid portion ground as thoroughly as possible in a mortar, put through a meat chopper, again covered with the original liquid, shaken several hours on a machine and filtered at the suction pump. The cake was then boiled one hour with water and again filtered with suction. The filtrates were now freed from mercury with hydrogen sulphide and the mercury-free acid fluids thus obtained were evaporated on the water bath under the electric fan to a small volume. Alcohol was then added from time to time and the evaporation was continued until the fluid was practically free from acid. The small residue (a few cubic centimeters) was now treated with solid sodium carbonate until there was no further evolution of carbon dioxide. A thick paste resulted and this was evaporated to dryness in vacuo over sulphuric acid at room temperature. The dried material was then powdered and extracted five times with boiling chloroform and the chloroform extracts were evaporated to dryness.

The residue from the chloroform was taken up in a little water and 10 per cent hydrochloric acid, filtered from a small amount of insoluble material, treated with solid mercuric chloride and warmed gently (40 to 50°). On cooling there separated a red tarry precipitate which was filtered off, suspended in water, decomposed with hydrogen sulphide, filtered from the mercuric sulphide and evaporated to dryness. This residue was later dissolved in water and brought up to a volume of 25 cc. and is called fraction I, posterior lobe. The corresponding extract of the anterior lobe was made up to 100 cc. and was called fraction I, anterior lobe.

The addition of dilute sodium hydroxide to the filtrate from the sticky mercury precipitate above described produced a white flocculent precipitate which was removed by filtration. This precipitate, as also the filtrate from it, was freed from mercury with hydrogen sulphide and the two fluids were then concentrated to a small volume on the water bath under the electric fan and the residues were freed from hydrochloric acid by repeated evaporation of added alcohol. The moist residues were thoroughly mixed with sodium carbonate and a small amount of water and were then dried in vacuo over sulphuric acid. The two residues were powdered and each was extracted several times with pure dry chloroform. As each of the two chloroform extracts

gave a strong Pauly reaction the two were combined and taken up in
very dilute hydrochloric acid. This solution was again treated with
mercuric chloride. This time only a little tarry, sticky precipitate was
thrown out. This was removed and discarded and dilute sodium
hydroxide solution was added to the clear filtrate until no further increase
of the precipitate was obtainable. The material was thus again
separated into two fractions—a mercury precipitate and the corre-
sponding filtrate, both of which were freed from mercury and evaporated
to dryness. As both fractions gave a strong Pauly reaction they were
combined and treated with silver nitrate and barium hydroxide accord-
ing to Kutscher's method for the separation of histidine from arginine;
the resulting "silver precipitate" and "silver filtrate" were both
decomposed with hydrochloric acid, filtered from silver chloride and
evaporated. The dry residue of each was extracted several times with
95 per cent alcohol, the alcohol removed and the residue dissolved in
water. The two residues were known as Fraction II, silver precipitate
of the posterior lobe, volume-30cc., and Fraction III, silver filtrate of the
posterior lobe, volume-27cc.

The corresponding extracts of the anterior lobe material were simi-
larly designated, Fraction II, silver precipitate of the anterior lobe,
volume-26cc. and Fraction III, silver filtrate of the anterior lobe,
volume-28cc.

*The chloroform soluble portions of the posterior and the anterior
lobes* were thus separated into three fractions as described in the
following table, in which also is given the volume of fluid to which
each fraction was made up.

	FRACTION I "MERCURIC CHLOR- IDE TAR"	FRACTION II SILVER PRECIPITATE	FRACTION III SILVER FILTRATE
	cc.	cc.	cc.
Posterior lobe........♦......	25	30	27
Anterior lobe...............	100	26	28

The following tracings show the relative activity for the uterus,
the arterial pressure and the bronchi of aliquot parts of most
of these fractions of the chloroform soluble material present
in the anterior and the posterior lobes. It will be seen that the
largest amount of active material was present in fraction I.
A voluminous precipitate of this character is bound to carry

down a large amount of a substance such as histamine, a substance which is ordinarily not precipitated in pure aqueous solution by the reagent used. All of the fractions obtained from the chloroform extract of the posterior lobe, as will be seen from the following tracings, contain a substance which acts similarly to histamine on the uterus, the arterial pressure, and the bronchi. The chloroform extract of the anterior lobe, on the other hand, gave up all of its histamine or histamine like substance to fraction I. It is of interest to note in this connection that fractions II and III of this lobe have an entirely negative reaction for histamine with p-diazobenzene sulphonate, and that these fractions were also almost entirely devoid of physiological action. Here it may also be stated that 'we have always made this test in the following manner in this laboratory. A small amount of either a dry or moist extract, or other product, is dissolved in a few drops of water, dry sodium carbonate in excess is added and then a minute, but appropriate, amount of the p-diazobenzene sulphonate solution is allowed to flow into this concentrated alkaline solution of the extract. If the chloroform soluble histamine body is present the characteristic deep red color of the histamine reaction is at once obtained. If now the contents of the test tube which give this postive reaction be diluted with water the deep red color gives place to a yellow tint and this occurrence may give rise to the belief that no histamine or histamine like substance is present, since a solution of histamine to which p-diazobenzene sulphonate has been added will retain a fine pink color on dilution with water. Working in company with Professor Abel I have recently satisfied myself that the change from the deep red solution to a yellow color on dilution with water in the reaction just described is due to foreign substances which themselves give an intense yellow color with p-diazobenzene sulphonate even in high dilution. Professor Abel and I have separated from a chloroform extract which was made from posterior lobes preserved with 2 per cent mercuric chloride and which behaved in the manner described when treated with p-diazobenzene sulphonate, a slightly impure histamine picrate which melted at 219° C. (uncorrected) and which maintained the

characteristic red or pink color when the reaction product with the Pauly reagent was diluted with water. This question of the presence of histamine in the pituitary extract will be treated at greater length by another hand in a later paper.

When the above pituitary fractions were analysed by Koessler and Hanke's (8) colorimetric method for estimating imidazole derivatives, using Methyl Orange-Congo Red solution as a standard, it was found in accordance with what has been said above that our highly dilute pituitary fraction developed a color which was much yellower than the standard. An *exact* comparison with the latter was therefore impossible, but the *intensities* of the colors of the solution under examination and of the standard solution (whose color equivalent had been determined by comparison with a solution of known strength of the acid phosphate of histamine) could be matched fairly accurately. The relative amounts of color-producing substance, calculated in terms of milligrams of the acid phosphate of histamine found in the various fractions of the chloroform soluble material from both the posterior and anterior lobes are given in the following table:

Relative amounts of histamine as determined by Koessler and Hanke's (8) method in terms of milligrams of the acid phosphate of histamine in the various fractions

	FRACTION I	FRACTION II	FRACTION III	TOTAL
Posterior lobe...............	2.62	0.55	0.67	3.84
Anterior lobe...............	1.07	0	0	1.07

It must be borne in mind that this table does not give the total yield of histamine or histamine-like substance, as equivalent fractional parts of the extracts, of which no account was kept, were used in numerous tests and furthermore a very considerable loss[2] of material occurs in the manipulations above described. Nevertheless, disregarding the great losses, the relative amounts of histamine in the two lobes of the pituitary gland may probably

[2] The chloroform extract at any given stage of the process represents only about 60 per cent of the chloroform soluble material. As this solvent was used several times considerable losses were unavoidable from this one cause alone.

be determined in this way with a fair degree of accuracy. I venture to think that the tests made on the bronchi of pithed dogs with known amounts of fraction I of the posterior lobe, when they are compared with the broncho-motor effects obtained with known amounts of the acid phosphate of histamine as in fig. 14 will support this conclusion.

DISCUSSION

It has been shown that aqueous extracts of the posterior lobe of the pituitary gland which have been prepared in a manner precluding bacterial action, and whose blood-pressure raising property has been abolished by means of mild hydrolysis with hydrochloric acid, contain a much larger amount of depressor and broncho-constrictor material than do corresponding extracts of the anterior lobe. Weight for weight of fresh lobe, extracts of the posterior lobe are about seven to eight times more depressant for the arterial pressure than similarly prepared extracts of the anterior lobe, as may be seen by an examination of the tracings of figure 1.

The broncho-constrictor action of such extracts of the posterior lobe is also many times greater than that of extracts of the anterior lobe. The relative activity of these extracts for the bronchioles has not yet been determined with any degree of accuracy, but an examination of the tracings given in figures 2, 3 and 4 would indicate that the ratio of broncho-constrictor activity is higher than 7 or 8 to 1 as was found for the blood-pressure lowering property of the two lobes. In other words, the posterior lobe contains a relatively larger proportion of broncho-constrictor substance, as compared with the anterior lobe, than it does of blood-pressure lowering substances. It is certainly worthy of note to find that aqueous extracts of the posterior lobe, prepared, as was emphasized above, in a manner which abolishes the blood-pressure raising property, and which furthermore precludes bacterial decomposition, should exhibit such a marked broncho-constrictor action. A certain part of this activity is transferable to chloroform as may be seen from the tracings of figures 5 to
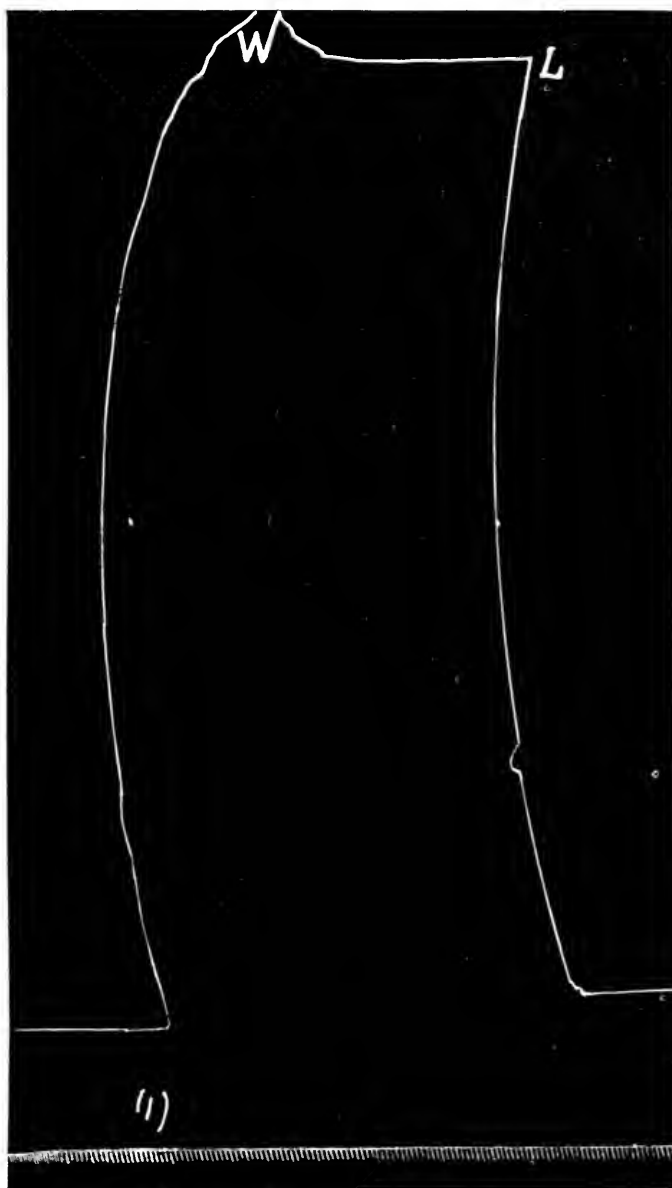
FIG. 5. ONE ENTIRE HORN VIRGIN GUINEA-PIG'S UTERUS

Same as used in figures 6 and 7. At *1* one drop (30 drops to 1 cc.) out of a total volume of 25 cc. of aqueous extract of chloroform extract—fraction I (mercuric chloride tar) of the posterior lobe. At *W*, weight was attached to the lever. At *L*, change to fresh Locke's solution. 30 cc. of solution were used in the uterine chamber.

13 inclusive. Lack of time prevented me from following out this point more fully, as otherwise I should have studied also the effect of the "B" substance of Abel and Nagayama on the bronchioles. The broncho-constrictor, blood-pressure lowering and oxytocic substance which passes into chloroform as is demonstrated in the tracings of figures 5 to 13 will probably be conceded to be histamine, in spite of the fact that the chemical evidence here offered for its identity with histamine is not confirmed by an actual isolation of the substance.
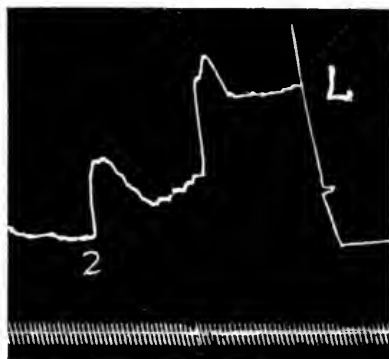
FIG. 6. ONE ENTIRE HORN VIRGIN GUINEA-PIG'S UTERUS

Same as used in figures 5 and 7. At *2* one drop out of a total volume of 100 cc. aqueous solution of chloroform extract of fraction I (mercuric chloride tar) of the anterior lobe. At *L*, change to fresh Locke's solution. 30 cc. solution were used in uterine chamber.

The blood-pressure raising substance, (the "A" substance of Abel and Nagayama) does not in its *native, uninjured* state induce broncho-constriction, at least not in any doses in which it has as yet been employed by us. I shall here give only one tracing[3] in illustration of this statement (fig. 15). The question whether broncho-constriction arises as a new property together with the blood-pressure lowering effect when this "A" substance

[3] This figure has been kindly supplied to me by Messrs. Lamson and Vermooten who were engaged in the study of the broncho-motor effects of the various pituitary principles.

is treated with dilute acid is now being studied. *I shall here anticipate a later paper from this laboratory far enough to state that when the "proteid—HgCl₂—precipitate" (see Abel and Nag-*
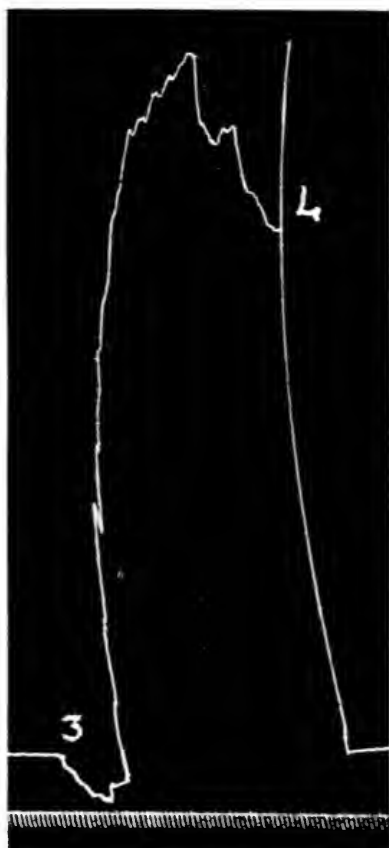
FIG. 7. ONE ENTIRE HORN VIRGIN GUINEA-PIG'S UTERUS

Same as used in figures 5 and 6. At *3* three drops out of a total volume of 100 cc. aqueous solution of chloroform extract of fraction I (mercuric chloride tar) of the anterior lobe. At *L*, change to fresh Locke's solution. 30 cc. of solution were used in uterine chamber.

ayama, pages 365 and 378) from which workers here are now preparing their most powerful pressor and oxytocic substance or substances is decomposed in the presence of a sufficient amount
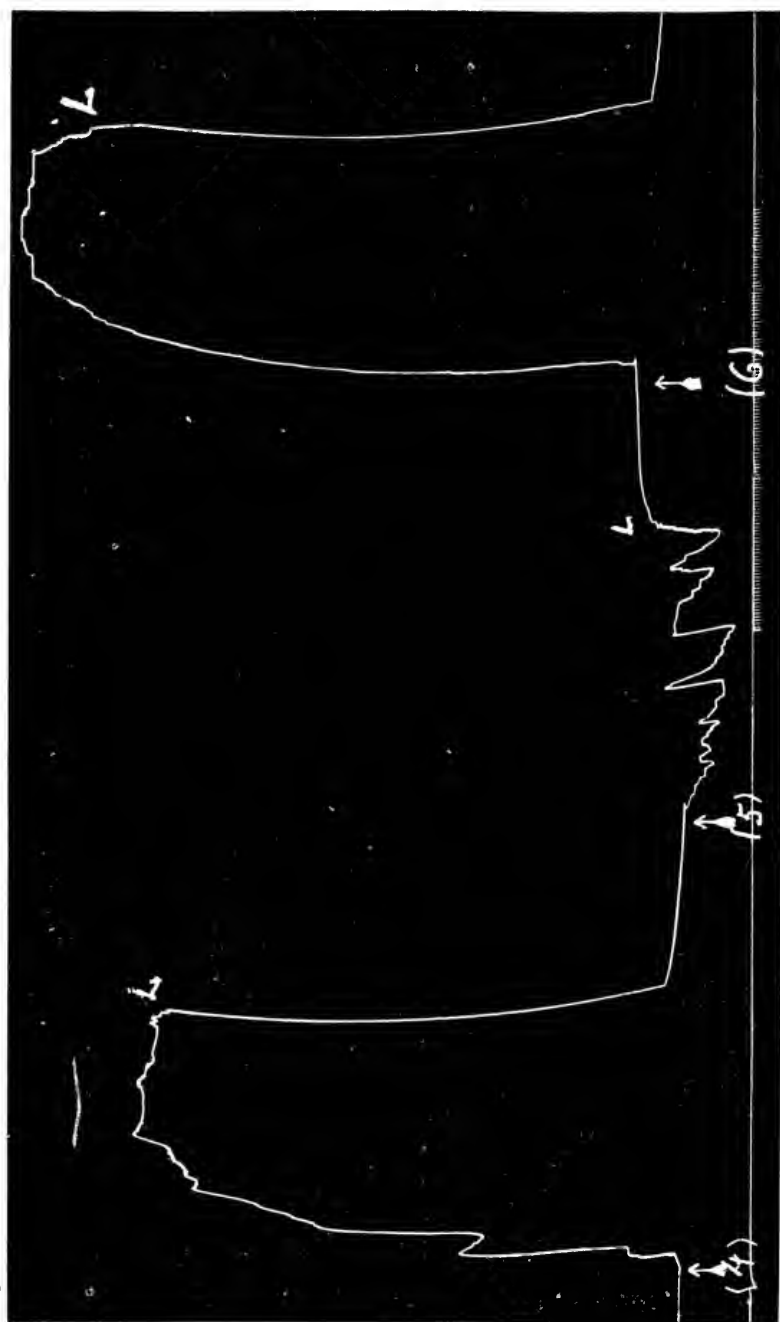
FIG. 8. ONE ENTIRE HORN VIRGIN GUINEA-PIG'S UTERUS

At *4* eight drops (30 drops to 1 cc.) of aqueous solution of the chloroform extract of fraction II (silver precipitate) of the posterior lobe. At *5* eight drops aqueous solution of the chloroform extract of fraction II (silver precipitate) of the anterior lobe. At *6* eight drops of aqueous solution of the chloroform extract of fraction II of the posterior lobe, same as at *4*. At *L, L* change to fresh Locke's solution. (30 cc. of solution were used in uterine chamber.)
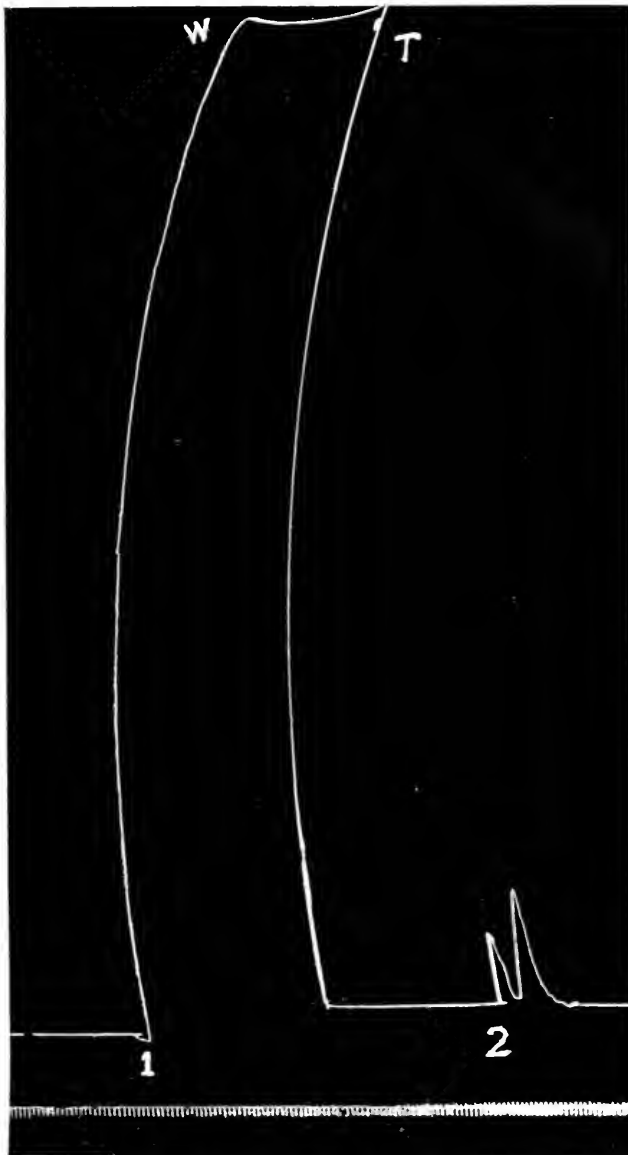
FIG. 9. ONE ENTIRE HORN VIRGIN GUINEA-PIG'S UTERUS

At *1* four drops (30 drops to 1 cc.) out of a total volume of 27 cc. aqueous solution of chloroform extract of fraction III (silver filtrate) of the posterior lobe. At *2* four drops out of a total volume of 28 cc. aqueous solution of chloroform extract of fraction III (silver filtrate) of the anterior lobe. At *W* a weight was attached to the lever. At *T* change to fresh Tyrode's solution. 30 cc. of Tyrode's solution in uterine chamber.

17

*of hydrochloric acid to abolish the blood-pressure raising property,
a decided broncho-constrictor action now for the first time makes
its appearance in conjunction with the newly appearing blood-
pressure lowering action.* The question whether one or both of
the newly acquired properties of the solution are really the results
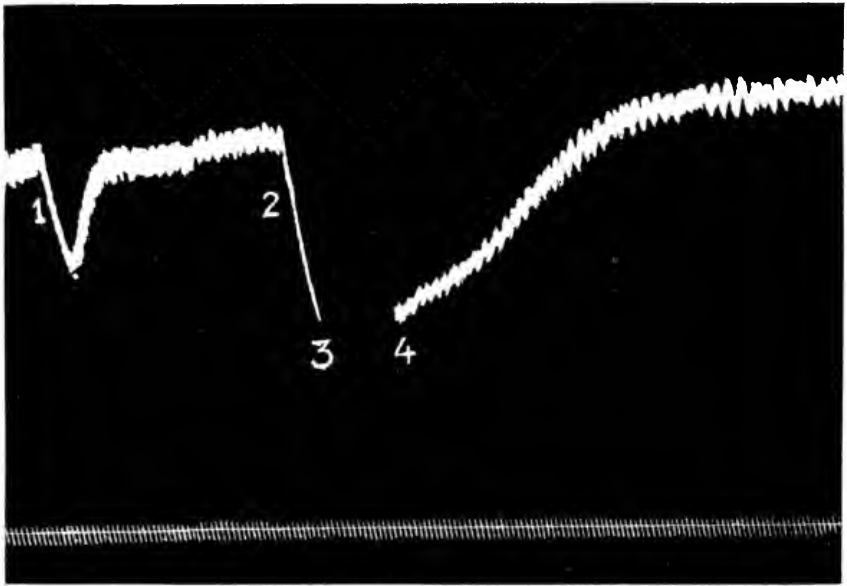
FIG. 10. MALE DOG, 5.8 KGM., PARALDEHYDE ANESTHESIA

Comparative effect on the arterial blood pressure of aqueous solutions of the
chloroform soluble material (fraction I) anterior and posterior lobes. At *1*
injected into the femoral vein 4 cc. out of a total quantity of 100 cc. of aqueous
solution of fraction I (mercuric chloride tar), anterior lobe. At *2* injected into
the femoral vein 4 cc. out of a total quantity of 25 cc. of aqueous solution of frac-
tion I (mercuric chloride tar) posterior lobe. At *3* the float of the manometer
was caught; the arterial pressure fell considerably below this point. As the
arterial pressure rose the mercury again came into contact with the float at *4*.

of an alteration of the pressor and oxytocic substance "A" must
remain an open one for the present.

In this connection the question may be raised whether any
share in the broncho-constrictor action of our extracts is to be
attributed to choline. This widely distributed substance has

been isolated by Engeland and Kutscher (9) from among the constituents of the pituitary gland. D. E. Jackson (10) says of the broncho-constrictor action of choline chloride:

With small doses I have obtained only slight effects, but with larger quantities I have observed a marked rise of blood pressure and a very extensive dilatation of the bronchioles in dogs . . . . in which the brain and medulla were destroyed by chloroform and the vagi nerves of which were sectioned.
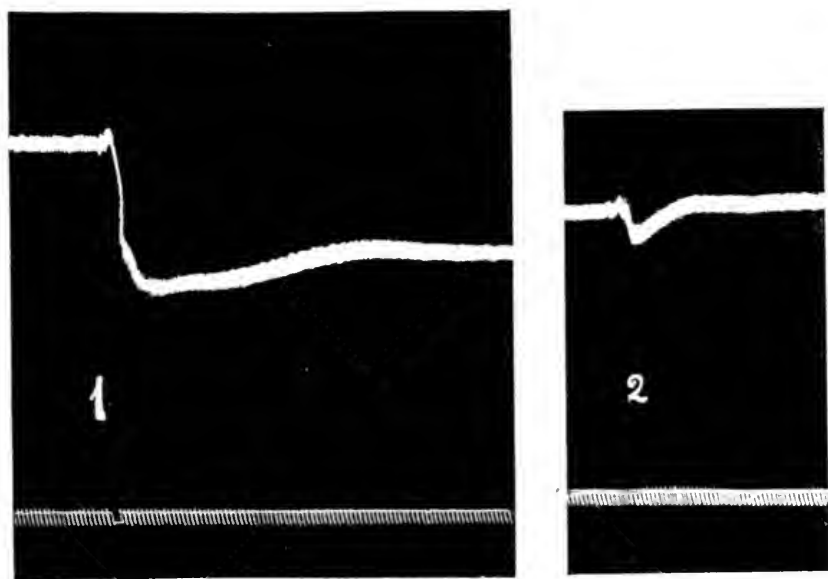
FIG. 11. DOG, MALE, 5.8 KGM., PARALDEHYDE ANESTHESIA (?)

Comparative action on arterial blood pressure of aqueous solutions of chloroform extract—fraction II (silver precipitate) of the anterior and posterior lobes. At *1* injection into the femoral vein of 1 cc. out of a total volume of 30 cc. aqueous solution of chloroform extract fraction II (silver precipitate) of the posterior lobe. At *2* injection of 10 cc. out of a total volume of 26 cc. of aqueous solution of chloroform extract—fraction II (silver precipitate) of the anterior lobe.

The tracings of lung volume of figure 16 show that under the conditions of my experiment doses of 5 and even 10 mgm. of choline chloride had no effect whatever on the bronchioles of a pithed dog weighing 6.9 kgm. It appears to be certain then

that we may exclude choline as one of the broncho-constrictor
agents of the pituitary extracts.

The chloroform extracts, however, of my posterior lobe prepara-
tions caused a very noticeable constriction of the bronchioles, as
may be seen in the tracing of figure 12. The change in lung
volume shown in this tracing is not as great as it should be for
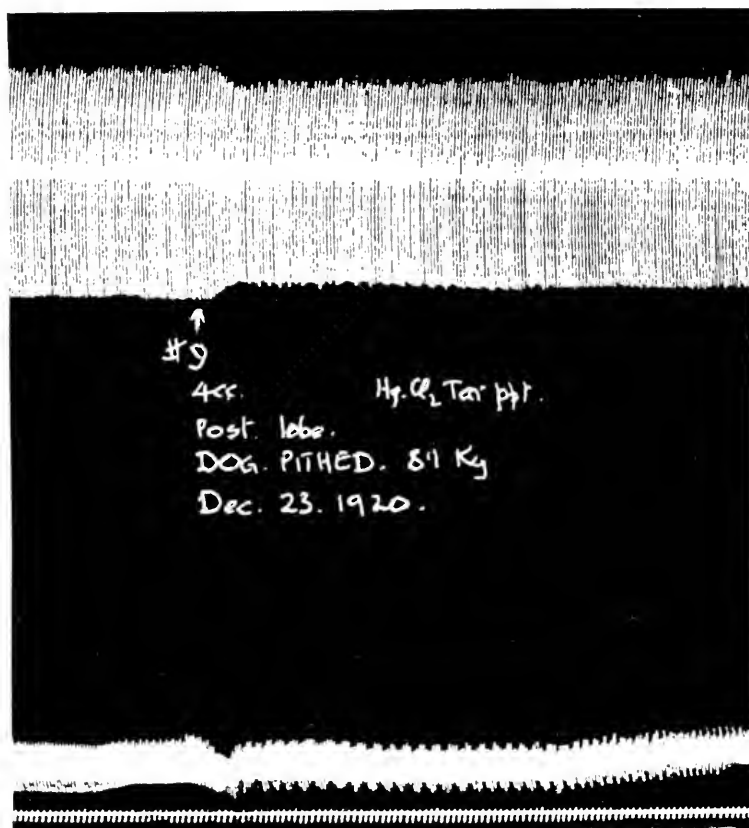
FIG. 12. EFFECT OF INJECTING INTO THE FEMORAL VEIN OF A PITHED DOG 4 CC.
OUT OF A TOTAL QUANTITY OF 25 CC. AQUEOUS SOLUTION OF THE CHLOROFORM
EXTRACT OF FRACTION I (MERCURIC CHLORIDE TAR) OF POSTERIOR LOBE

The 25 cc. contained the equivalent of 2.62 mgm. of β-I acid phosphate, and
the above tracing is what might be expected from the injection of 0.4 to 0.5 mgm.
of β-I acid phosphate.

the reason that my apparatus was not arranged to respond to minute doses of a broncho-constrictor agent. The minimum effective dose for the acid-phosphate of histamine in this particular experiment was larger than 0.2 mgm. as may be seen by an exami-
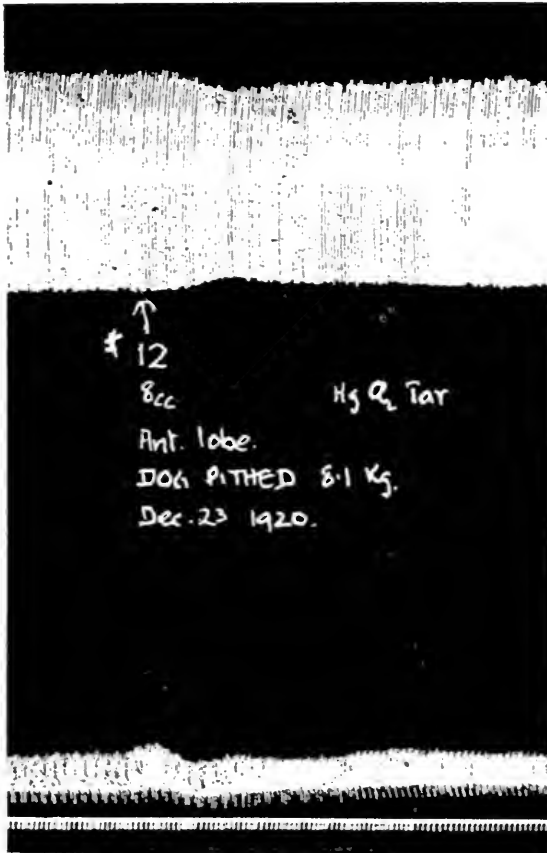
FIG. 13. EFFECT OF INJECTING INTO THE FEMORAL VEIN OF A PITHED DOG 8 CC OUT OF A TOTAL QUANTITY OF 100 CC. AQUEOUS SOLUTION OF THE CHLOROFORM EXTRACT OF FRACTION I (MERCURIC CHLORIDE TAR) OF ANTERIOR LOBE

nation of figure 14. I feel confident that the amount of bronchial constriction induced by 4 cc. out of a total of 25 cc. of the chloroform extract as shown in the tracing of figure 12 is equivalent to that which would have been obtained from the injection of at

least 0.5 mgm. of the acid-phosphate of histamine. The fractions (II) and (III) of the chloroform extract also gave definite evidence of broncho-constrictor action, though this was naturally less pronounced in the case of these fractions, as smaller quantities of the extract were injected.
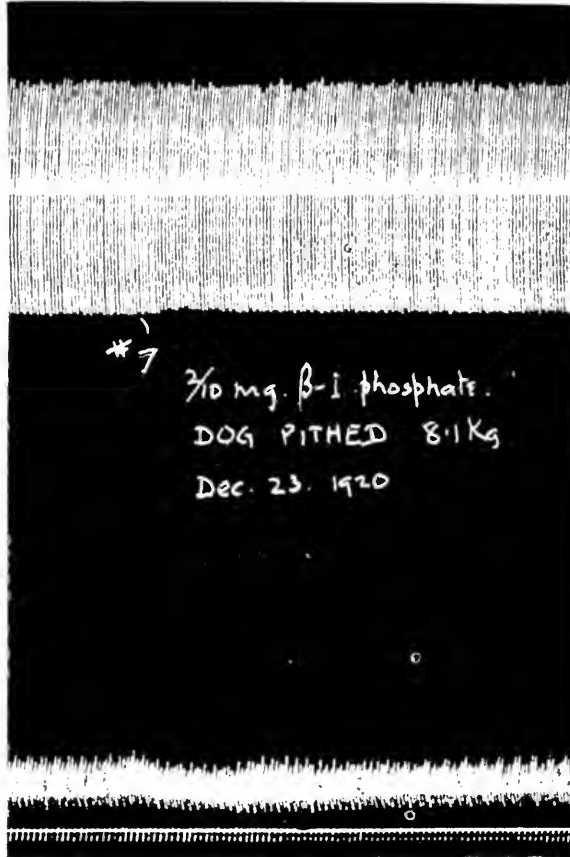
FIG. 14. SHOWS THAT THE APPARATUS EMPLOYED FOR THE PREPARATION OF FIGURE 12 IN WHICH THE CHLOROFORM EXTRACT OF THE POSTERIOR LOBE WAS SHOWN TO CONSTRICT THE BRONCHIOLES, WAS NOT SET TO REGISTER SMALL ALTERATIONS IN LUNG VOLUME

0.2 mgm. β-I acid phosphate ordinarily gives a very pronounced broncho-constriction. It is to be noted that the above injection preceded the injections of figures 12 and 13.
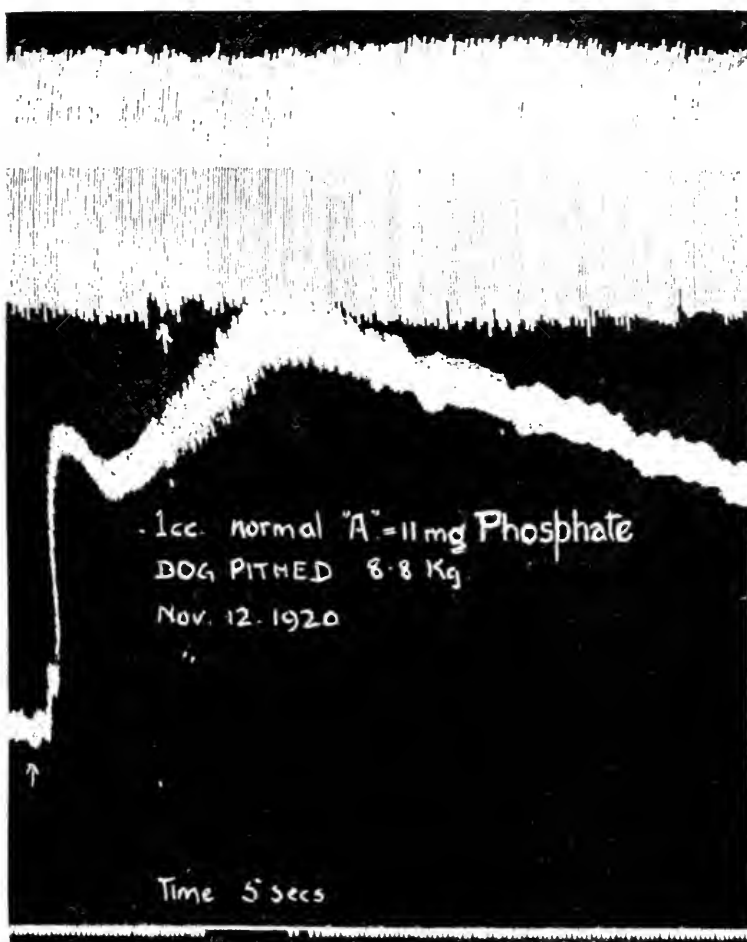
FIG. 15. EFFECT OF INJECTION INTO THE FEMORAL VEIN OF A PITHED DOG 1 CC.
SOLUTION OF PRESSOR AND OXYTOCIC PHOSPHATE "A" = 11 MGM. PREP-
ARED BY DECOMPOSING THE "PROTEID-HgCl₂-PRECIPITATE" OF ABEL
AND NAGAYAMA (THIS JOURNAL, XV, 365 AND 378, 1920.

It must be noted that the registering points for the lung volume and the blood
pressure were not in line. The arrows in the tracings indicate the respective
positions at the time of injection. It will thus be seen that the injection of a
powerful solution of "A" has no effect on the bronchioles, unless it be a slight
dilatation. Neither in this case, nor in many similar experiments have we
obtained a constriction of the bronchioles from the unaltered pressor substance.
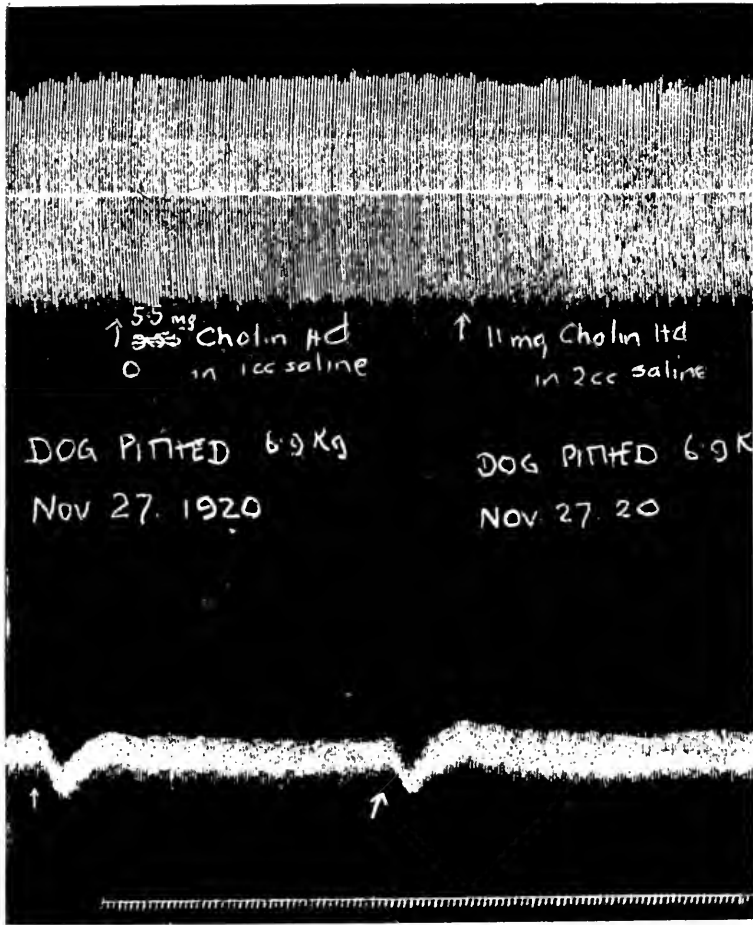
FIG 16. SHOWS THE ENTIRE ABSENCE OF BRONCHO-MOTOR ACTION OF 5.5 AND 11 MGM. OF CHOLINE CHLORIDE INJECTED INTO THE FEMORAL VEIN OF A PITHED DOG

SUMMARY.

1. Simple aqueous extracts of the posterior and anterior lobes of the pituitary gland prepared in such a way as to abolish their blood-pressure raising properties and to preclude all chance of bacterial activity were found to possess blood-pressure lowering and broncho-constrictor properties.

2. Extracts of this kind made from the posterior lobe of the gland are from seven to eight times more depressant for the arterial pressure than are similar extracts of the anterior lobe, equal weights of the two lobes being used as the basis of comparison.

3. The broncho-constrictor action of extracts of the posterior lobe is very marked in comparison with that exhibited by extracts of the anterior lobe and the ratio of the activity of equivalent extracts of the two lobes is certainly greater than 8 : 1.

4. Chloroform takes up from a properly prepared and dried extract of sterile posterior and anterior lobes of the pituitary gland a determinable amount of a substance which acts like histamine on the arterial pressure, the uterus and the bronchi. The posterior lobe yields to chloroform about twenty times more of this substance than does the anterior lobe, weight for weight of fresh material.

5. The substance which passes into chloroform and which has the pharmacodynamic action of histamine is thought to be this latter compound, because of its behavior during the chemical manipulations to which it was subjected in my work, as also on the ground of its physiological properties.

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ON THE PITUITARY ACTIVE PRINCIPLES AND HISTAMINE

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I. INTRODUCTORY

In an earlier paper one of us described a method (1) for obtaining the pressor and oxytocic principles from the posterior lobe of the pituitary gland in a state of partial purification, and of effecting a partial separation of the two kinds of activity. Evidence was then presented against the identification, which Abel and Kubota (2) had suggested, of the oxytocic principle with histamine. Further objections to this identification have been put forward by Jackson and Mills (3) and by Hanke and Koessler (4), and in a more recent paper by Abel and Nagayama (5) it has been definitely withdrawn. Abel and Nagayama recognise that the method adopted by Abel and Kubota for extracting the principle from the pituitary substance was so drastic as to destroy the whole of the specific activity in the portion worked up, and that a larger part of the active principles was left in the first mercurial precipitate; this latter, discarded in the earlier process, is now used by Abel and Nagayama as a source of a specially active preparation. At the same time they do not altogether abandon the idea of a connexion between histamine and the pituitary active principle; it is admitted that it is not itself the specific principle, but evidence is put forward for its appearance during the hydrolytic destruction of that principle. If clear evidence could be produced of the formation of histamine from the destruction of any of the pituitary principles, the fact would be of greater significance than any yet known concerning the chemical nature of these substances. We

27

were led by these considerations to repeat and extend one of Abel and Nagayama's observations which seemed to point most clearly in this direction, and the results, though purely negative, seem worthy of record. We take the opportunity of recording some further observations on the effect of proteoclastic ferments on the active principle.

II. HYDROLYSIS OF PITUITARY ACTIVE PRINCIPLES

1. Is histamine usually present in pituitary extracts?

Abel and Nagayama, while they handsomely acknowlege the cogency of Dudley's evidence against the identity of the pituitary oxytocic principle and histamine, criticize his statement that "there is no detectable quantity of histamine present in extracts of the infundibular material." It must be remembered that Dudley's statement was made in answer to the allegation of identity, and we believe that it will have been generally understood in the sense intended, namely, as meaning that, in concentrations far beyond those giving the maximum of the specific oxytocic action, pituitary extracts failed to show any activity which could be attributed to histamine, so that histamine, so far from being the active principle, made no perceptible contribution to the oxytocic action as ordinarily detected. In this sense we still believe that the statement is perfectly accurate. Abel and Nagayama, however, interpret the statement in a more absolute sense, and produce evidence which they regard as indicating the presence of histamine in the ordinary commercial infundibular extracts, and even, though apparently in much smaller amount, in extracts prepared with special care. It will be worth while to consider briefly the quantitative meaning of their evidence.

It should be noted, in the first place, that their evidence for the presence of histamine is that dry chloroform removes, from the extract dried with sodium carbonate, a substance which causes a fall of arterial blood-pressure in the cat or dog. Let us assume that this evidence is sufficient for identification, and take the figures from the example in which Abel and Nagayama's

physiological comparison is fairly complete. From 80 cc. of Burroughs, Wellcome and Company's "Infundin" they obtained a chloroform extract, which was dissolved in 5.8 cc. of water. Of this solution 0.5 cc. produced a fall of arterial pressure approximately equal to that produced by 0.1 mgm. of histamine diphosphate. On this calculation the 80 cc. yielded histamine equivalent to 1.16 mgm. of the diphosphate, i.e., a little more than 0.4 mgm. of histamine base. The total net weight of the chloroform extract was 3.8 mgm., so that the extraction had no strong specificity; if the activity was due to histamine, this was mixed with about eight times its weight of inert material. We will assume, however, the correctness of Abel and Nagayama's attribution of the activity to histamine, and of their further calculation that the total amount obtainable, if extraction were complete, would be equivalent to 2 mgm. of the diphosphate, giving 0.025 mgm. of diphosphate, or less than 0.01 mgm. of the base per cubic centimeter of the "Infundin." Let us take it as 0.01 mgm. The ordinary testing dilution for such an extract on the isolated uterus is obtained by adding about 0.003 to 0.005 cc. to about 100 cc. of Ringer's solution; a higher concentration would produce a supramaximal effect. The concentration of histamine thus produced in the bath on the most favorable assumption, could not be more than 1 in

$$\frac{200 \times 100 \times 1000}{0.01} = 1 \text{ in } 2000 \text{ millions.}$$ This is far beyond

the range in which histamine produces any perceptible effect on the isolated uterus of the guinea-pig, so that, if we accept Abel and Nagayama's estimate, histamine is not present, even in a commercial infundibular extract, in sufficient concentration to complicate the recognition and evaluation of the specific oxytocic principle. In a carefully prepared extract, more comparable with that with which Dudley worked, they found evidence of not more than one-fifth of this amount.

With the question, however, as to whether histamine itself, in minute amount, is actually present in ordinary infundibular extracts, or even in the substance of the fresh gland, we are not directly concerned. The whole interest of the matter for

us lies in Abel and Nagayama's suggestion that histamine appears with especial readiness in the extracts of this material when subjected to mild hydrolysis, and the implication that the apparent formation of histamine, as the specific principle is hydrolysed, has some significance for the constitution of the latter.

2. Is histamine formed by hydrolysis of pituitary extract?

Abel and Nagayama state that, while the pressor action of a pituitary extract is rapidly and completely abolished by brief acid hydrolysis (boiling with 0.5 per cent HCl for half an hour), the oxytocic activity is not completely abolished, but reduced to about 20 per cent of the initial activity, below which level even prolonged hydrolysis does not reduce it. Of this remnant of oxytocic activity they find that about one-fifth is due to a substance extracted by chloroform after drying with sodium carbonate, and regarded on that account as histamine, and four-fifths to a substance insoluble in chloroform, but soluble in strong alcohol, which they call the "histamine-like substance," finding that it "behaves in respect to both blood-pressure and uterus exactly like histamine." So that an extract subjected to sufficiently long acid hydrolysis to reduce the activity on the uterus to about one-fifth, should show a similar proportion between depressor effect on the blood-pressure and stimulant action on the uterus to that shown by histamine; and with further hydrolysis neither should change. We put this interpretation of Abel and Nagayama's findings to the test of experiment.

Experiment I. This preliminary experiment was made with a more dilute extract than that used by Abel and Nagayama. We used a "1 per cent extract," purified with colloidal ferric hydroxide, as described by Dudley (1). The dried infundibular material was obtained from the Hollister-Wilson Laboratories, Chicago, and we are indebted to Mr. B. K. Hollister for his coöperation in enabling us to obtain a good supply of this material. The following preparations were made:

O = the original, purified "1 per cent extract."

A. To 20 cc. of O were added 20 cc. of 1 per cent HCl. The mixture, containing 0.5 per cent HCl, was boiled under a reflux condenser for thirty minutes. It was then cooled, neutralized with soda, and made up to 60 cc., so that 3 cc. A represent 1 cc. O.

B. Was made precisely as A, except that the boiling with 0.5 per cent HCl was continued for six hours.

The solutions A and B were tested on the blood pressure of a cat under ether, in comparison with a standard solution of histamine, and on the isolated uterus of a guinea-pig in comparison with both O and histamine. After a number of preliminary trials the following equivalents were obtained and confirmed.

1. Depressor action on cat's arterial pressure. 5 cc. A = 5 cc. B = 0.002 mgm. of histamine. So that 1 cc. A or B = 0.0004 mgm. histamine, and 3 cc. of A or B, representing 1 cc. of O = 0.0012 mgm. of histamine.

It may be noted at once that this depressor action corresponds to that of a concentration of histamine which would not have anything approaching one-fifth of the activity of O on the uterus. The dose of O producing a maximal uterine contraction in 100 cc. of Ringer's solution is about 0.02 cc.; 0.3 cc. of A or B (i.e., 3 × 5 × 0.02 cc.) corresponds in depressor action to 0.00012 mgm. of histamine—a dose far below the threshold of stimulation for the uterus in 100 cc.

2. Tests on isolated uterus. O was diluted 60 times for testing, A and B being used undiluted. Each cubic centimeter of these therefore represents 20 cc. of the dilution of O. The following comparisons were obtained:

$$1.5 \text{ cc. O (dil.)} \quad <0.8 \text{ cc. A.}$$
$$>0.7 \text{ cc. A.}$$
$$1.2 \text{ cc. O (dil.)} \quad <0.7 \text{ cc. A.}$$
$$>0.5 \text{ cc. A.}$$

From either 1 cc. O (dil.) = approximately 0.5 cc. A, i.e., 0.05 cc. O = 0.5 cc. A, when both are diluted equally. A has retained one-tenth of the original activity on the uterus; but

a solution of histamine, equidepressor with A, has no percep-
tible effect on the uterus in this dosage.

This activity on the uterus was not, as in Abel and Nagayama's
experiment, stable to further hydrolysis. For 0.7 cc. of B hardly
affected the uterus at all, and 3.5 cc. of B produced a much
smaller effect than 0.7 cc. of A. Larger doses could hardly
be tested, owing to the high salt content. It may safely be
said, however, that B had less than one one-hundredth of the
activity of O in equivalent dilution. Even so its activity on
the uterus was greater than that of an equidepressor dose of
histamine.
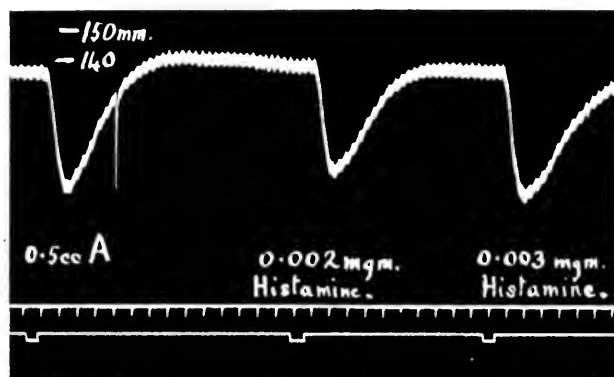
Fig. 1. See Experiment II. Blood Pressure of Cat Under Ether

Experiment II. For experiment II we used a stronger origi-
nal extract, so as to imitate more closely the conditions of
Abel and Nagayama's experiments. The "1 per cent extract"
of dried infundibulum, purified by precipitation with colloidal
ferric hydroxide, was concentrated by evaporation to one-tenth
of its volume, giving a "10 per cent extract." The following
preparations were made and designated by key-letters.

O = the original 10 per cent extract.

A. To 20 cc. of O were added 20 cc. of 1 per cent HCl, and
the mixture boiled under reflux condenser for thirty minutes.
It was then neutralized and made up to 60 cc., so that 3 cc.
A = 1 cc. O.

B. Similar to A, but boiling was continued for six hours, 3 cc. B = 1 cc. O.

Test on blood-pressure. The mixtures were compared by intravenous injection into two cats under ether, the depressor effects being matched with those of minute doses of histamine. Identical matches were obtained in the two experiments. 0.5 cc. of A or B corresponds to 5 cc. of the preparation so lettered in experiment I; and 3 cc. of A or B again represent 1 cc. of the original extract.

<div style="text-align:center">

0.5 cc. A = 0.002 mgm. of histamine.

0.5 cc. B = 0.003 mgm. of histamine.

</div>

Cat A   Cat B
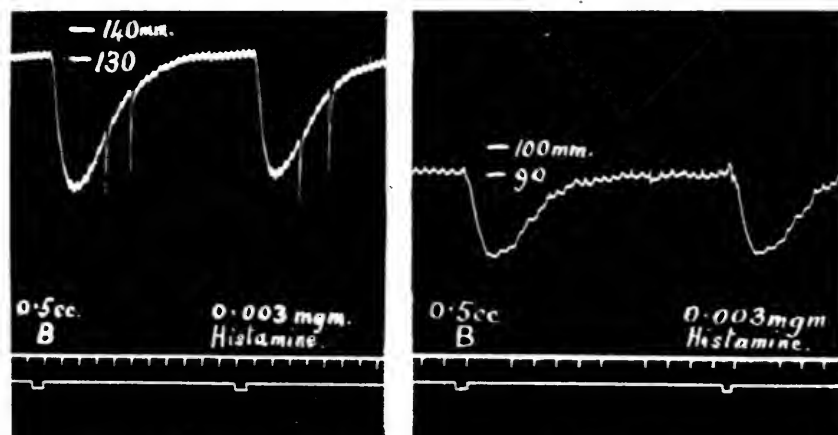
FIG. 2. SEE EXPERIMENT II.   BLOOD PRESSURE OF CAT UNDER ETHER

The hydrolytic changes, on the 10 per cent extract, have apparently proceeded somewhat more slowly than in experiment I, where a 1 per cent extract was used. B, the extract boiled with 0.5 per cent HCl for six hours, has distinctly a more powerful depressor action than A, which was similarly treated for thirty minutes. The depressant action of A is the same, with corresponding doses, as that of A in experiment I; B is 50 per cent stronger than the B of experiment I. There is evidence, therefore, of the progressive formation of some small amount of depressor substance by hydrolysis.

Tests on the isolated uterus of the guinea-pig. Doses of the original extract were found which gave slightly greater and slightly smaller effects than a given dose of the preparation under test, and finally a match was made as exactly as possible with equally stimulant doses. The original extract was diluted, for convenience of measurement, 200 times, while the doses of the different preparations were diluted when necessary to a strength giving a convenient volume of dosage. For purposes of calculation all are expressed in terms of the same equivalent
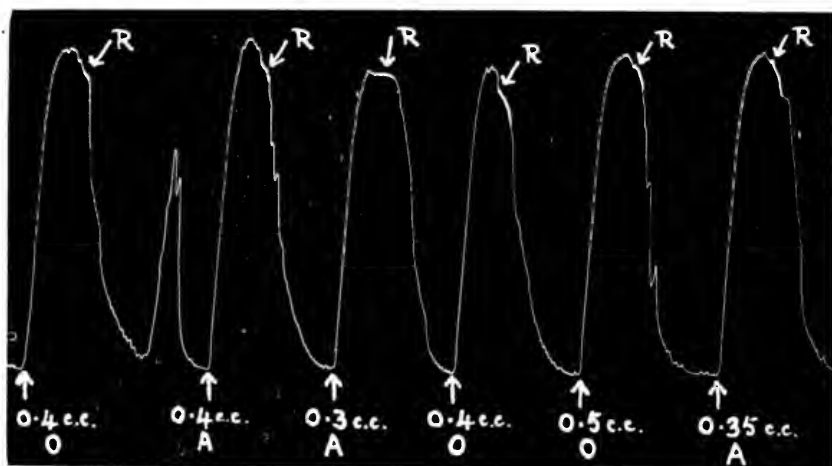
Fig. 3. See Experiment II

O = Original extract diluted 200 times.   A = Solution A diluted 10 times, i.e., to 30 times original volume.

dilution—600 times the original; but this does not mean, of course, that the indicated volumes were added; A was diluted for testing to a volume representing 30 times the original, and B was added to the bath without dilution beyond that involved in its preparation; i.e., equivalent to O three times diluted. The following were the results.

1.5 cc. O = 7 cc. A.   Ratio of activities 4.7:/1
1.2 cc. O > 200 cc. B.
Approximately = 260 cc. B.   Ratio of activities 217:/1

In this case the residue of activity on the uterus after thirty minutes' acid hydrolysis (A) is approximately one-fifth, as found by Abel and Nagayama; starting with a stronger extract, therefore, we obtain a result on this point corresponding with theirs. With six hours hydrolysis, on the other hand, we again fail to confirm their finding of no further decline; on the contrary, we find that the action on the uterus is now less that one two-hundredth of that of the original extract.
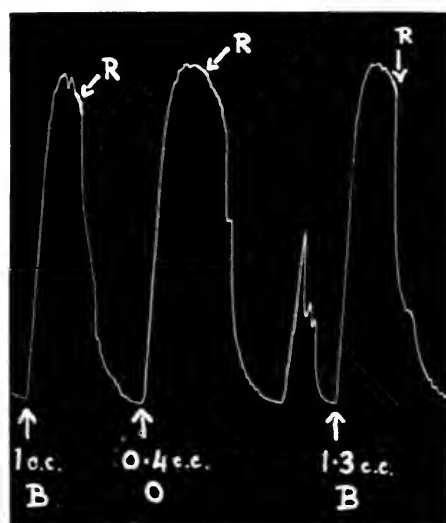
FIG. 4. SEE EXPERIMENT II

O = Original extract diluted 200 times.   B = Solution B not further diluted; i.e, at 3 times original volume.

Again there is the most complete discrepancy between the histamine-like depressor actions of these two preparations A and B, and their stimulant actions on the isolated uterus. A shows 50 per cent less depressor action than B, possibly on account of the survival of a trace of the specific pressor principle; A, on the other hand shows between 40 and 50 times the stimulant action on the uterus which B exhibits, and the conclusion is scarcely avoidable that it owes this superiority to the survival of a part of the specific oxytocic principle. Abel and

Nagayama's conclusion that this remnant of one-fifth of the normal activity is due to histamine and a substance identical in action with histamine cannot possibly be applied to our results. The dose of A producing nearly maximal uterine contraction was 0.35 cc. This would be equivalent in depressor action to $\dfrac{0.35 \times 0.002}{0.5}$ mgm. $= 0.0014$ mgm. of histamine, which would produce an imperceptible, or barely perceptible reaction with the uterus under the conditions of the test. Even B, again, which shows less than one two-hundredth of the original activity on the uterus, has still a stronger oxytocic action in proportion to its depressor action, than histamine has.
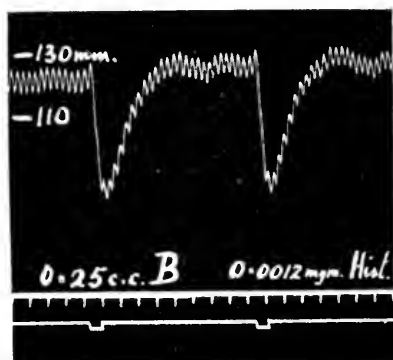
FIG. 5. SEE EXPERIMENT III. BLOOD PRESSURE OF CAT UNDER ETHER

Experiment III. This experiment was conducted on similar lines to those of experiment II. A "10 per cent extract" was made, but the purification by means of colloidal ferric hydroxide was omitted. The results obtained on hydrolysing this extract with 0.5 per cent HCl did not differ materially from those of experiment II, and we include here solely the tracings illustrating the fact, already mentioned, that the uterine stimulant action of B (prepared exactly as B of experiment II), slight as it is, is still much greater than that of an equivalent dose of histamine as measured by blood-pressure equation.

Figure 5 shows that 0.25 cc. B has an effect on the blood-pressure of cat equal to that displayed by 0.0012 mgm. histamine. So that 1 cc. of B is equivalent in depressor action to 0.0048 mgm. of histamine.

Figure 6 shows the effects of 1 cc. of B, and of the equide-pressor dose of histamine on the isolated uterus of the guinea-pig. It will be seen that the effect of B is definitely the greater.

So far, then, the evidence of these experiments does not in any way support the statement of Abel and Nagayama that the specific active principles are completely destroyed by brief hydrolysis with 0.5 per cent HCl, and that the action on the uterus still remaining is due to histamine and histamine-like
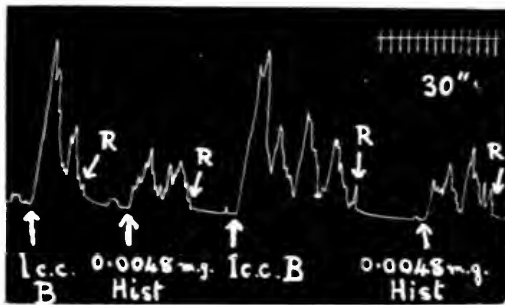
FIG. 6. SEE EXPERIMENT III

substances, largely produced by the hydrolysis, and stable to long continued treatment of the same kind. On the contrary, the natural conclusion from our results would be that both the pressor and oxytocic specific activities of the pituitary extract are destroyed by boiling with 0.5 per cent HCl, but that the process is not completed in thirty minutes, in which period only about four-fifths of the oxytocic principle has been de-stroyed. Further hydrolysis, continued for six hours, destroys most of the remaining one-fifth.

Our evidence does not enable us to decide definitely whether the small amount of activity on the uterus, then still detectable, is partly due to traces of the specific active principle which have

still escaped destruction, or wholly to non-specific substances
of the peptone or histamine type. We can only state that some-
thing is present which has a larger ratio of oxytocic to depressor
action than histamine, and that this would be explained by
the not improbable supposition that a trace of specific activity
remains.

We attempted to discover whether any of the depressor and
oxytocic activities left after prolonged acid hydrolysis were
due to histamine itself by subjecting the preparation to further
hydrolysis with boiling normal alkali. Both kinds of activity
can be abolished by such treatment, but this cannot be regarded
as sound evidence against the presence of traces of histamine,
since we found that, when histamine was added to preparation
B in a proportion of 0.001 mgm. per cubic centimeter, the ac-
tivity due to this, in addition to that previously present, mostly
disappeared when the mixture was boiled for an hour with
normal NaOH. We are not in a position to explain this dis-
appearance of histamine; possibly in the form of free base
it is adsorbed by inert colloidal constituents of the extract,
its activity becoming thereby masked. We mention the matter
chiefly because such apparent instability to alkali has been
regarded by some workers—Stern and Rothlin (6)—as an in-
dication that a substance is not histamine; a conclusion which
is evidently not justifiable when high dilutions and complex
extracts are in question.

While we must leave it as an open possibility that some part
of the action on the uterus, persisting after prolonged acid hy-
drolysis, may be due to histamine itself, we must again emphasize
the fact that the position, as it is left by our observations, is
very different from that indicated by Abel and Nagayama.
They found a persistent activity on the uterus about one-fifth
as great as that of the original extract, and attributed one-fifth
of this, again, corresponding to one twenty-fifth of the original
activity, to histamine. We find that the total activity on
the uterus, after six hours hydrolysis, is less than one
two-hundredth of the original, and on the same propor-
tional basis, the part of this due to histamine would be about

one-thousandth of the original activity. The action on the
uterus is, as we have indicated, probably intensified by per-
sistent traces of the specific principle. The estimate by means
of the blood-pressure is, therefore, more suitable for indicating
the possible content of histamine, on Abel and Nagayama's
basis. We found a depressor action equivalent to that of 0.006
mgm. of histamine per cubic centimeter of hydrolysed extract,
at thrice the initial dilution. Reducing this to the original
volume, and allowing the possibility of one-fifth being due
to histamine itself, we get a histamine yield of $\dfrac{0.006 \times 3}{5} =$

0.0036 mgm. from 1 cc. of 10 per cent extract. We should need
to use 1 litre of the extract to obtain 3.6 mgm. of histamine.
The project of chemical indentification of such minimal traces
in this extremely valuable material becomes wholly imprac-
ticable; nor could we regard even a clear demonstration of the
presence of histamine, in such quantities, in the hydrolysed
pituitary extract as having any necessary bearing on the nature
of the specific principles. As the evidence stands, we do not
think that the presence of even this trace of histamine is sat-
isfactorily established.

III. ACTION OF EREPSIN AND PAPAIN ON THE PITUITARY UTERINE STIMULANT

It has previously been shown that pepsin has no action on
the uterine stimulant, while trypsin destroys it with great
rapidity.

It was therefore considered to be of some interest to examine
the effect of other proteolytic enzymes on this substance.

Erepsin

The demonstration of the action of this enzyme was attended
with some difficulty owing to the feebleness of the ordinary
preparations.

One hundred grams of mucosa from a dog's intestine, which
had been thoroughly washed, were ground up with sand and

extracted with 200 cc. tap water to which had been added 5 cc.
1 per cent Na_2CO_3 and a little $CHCl_3$. After standing in the
cold room for four days the mixture was strained through muslin
and the filtrate was shown to hydrolyse peptone.

A "1 per cent extract" of dried pituitary infundibulum was
made as described in a previous paper (1). The "1 per cent
extract" was diluted with one volume of water and this solution
with three volumes of 0.1 per cent Na_2CO_3. To 5 cc. portions
of this solution were added: (1) 5 cc. erepsin preparation + 5
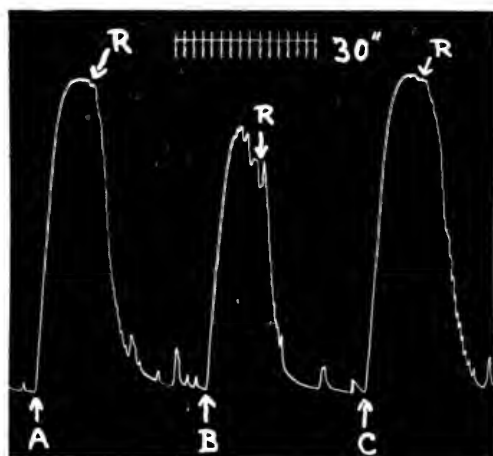
FIG. 7. EFFECT OF EREPSIN ON OXYTOCIC PRINCIPLE

A = 0.5 cc. solution (2); B = 0.5 cc. solution (1); C = 0.5 cc. of original
"1 per cent extract" 20 times diluted.

cc. 0.1 per cent Na_2CO_3, and (2) 5 cc. erepsin boiled + 5 cc.
0.1 per cent Na_2CO_3. The tubes were then incubated at 37°C.

Only very slight diminution of activity in (1), tested on the
isolated uterus of the virgin guinea-pig, was detectable in 4
hours, but after 24 hours the activity of solution (1) had en-
tirely disappeared, whilst that of (2) remained unimpaired.

It is known that bacterial action destroys the oxytocic principle
very rapidly, and although $CHCl_3$ was present in the erepsin
preparation used, it was thought desirable to make another

experiment in an attempt to demonstrate the action of erepsin in a shorter time.

One per cent extract was diluted five times with Na_2CO_3 solution of such a strength as to give a 0.2 per cent concentration of the salt. To 5 cc. of this solution were added: (1) 15 cc. erepsin solution, and (2) 15 cc. erepsin boiled solution. After three hours at 37°C. the activity of solution (1) was appreciably smaller than that of (2).

Erepsin, then, destroys the activity of the uterine stimulant slowly.

Papain

A commercial sample of this enzyme was shown to digest fibrin in $\frac{N}{10}$ and $\frac{N}{20}$ HCl.

20 cc. of "1 per cent extract" were diluted to 50 cc. with water, 1 gram papain was dissolved in 50 cc. water and filtered.

The following mixtures were then incubated at 37°C.:

(1) 5 cc. dil. "1 per cent extract" + 5 cc. papain solution + 10 cc. $\frac{N}{5}$ HCl.

(2) 5 cc. dil. "1 per cent extract" + 5 cc. water + 10 cc. $\frac{N}{5}$ HCl.

(3) 5 cc. dil. "1 per cent extract" + 5 cc. papain solution + 10 cc. $\frac{N}{10}$ HCl.

(4) 5 cc. dil. "1 per cent extract" + 5 cc. water + 10 cc. $\frac{N}{10}$ HCl.

After nineteen hours (1) and (2) were neutralized with equal volumes of appropriate Na_2CO_3 solutions, so that the final strength of the solutions was "1 per cent extract" diluted twenty times. The solutions were tested against each other and against "1 per cent extract" diluted twenty times on the isolated uterus of the virgin guinea-pig. It was found that in (1) and (2) about 20 per cent of the activity had been lost; the loss, being equal

in the two cases, must be attributed to the action of the hydro-
chloric acid.   The enzyme had had no effect.   After forty-three
hours solutions (3) and (4) were tested, and again it was found
that the enzyme had exerted no action on the uterine stimulant.

It was also shown by testing solutions (1) and (2) on the blood
pressure of a cat that the pressor principle of the pituitary
extract was not affected by papain.

Papain, therefore, in acid solution has no effect on the oxytocic
and pressor principles of the pituitary gland.

SUMMARY OF CONCLUSIONS

1. The specific pressor and oxytocic actions of the pituitary
extract are destroyed by boiling with 0.5 per cent HCl (con-
firming Abel and Nagayama).

2. The oxytocic activity is reduced to about one-fifth in
thirty minutes, as Abel and Nagayama found.   In contrast
to their observation that this remainder is stable to further
hydrolysis, we find that after 6 hours less than one two-hun-
dredth remains.

3. Even after six hours the extract has a stronger oxytocic
action than a dose of histamine of equal depressor action.

4. Histamine, if present at all, occurs in such minute amounts
that its chemical identification is impracticable, and there seems
no reason at present for suggesting a relation between histamine
and the specific action of the extract.

5. The specific oxytocic principle is slowly destroyed by erep-
sin, but neither it nor the pressor principle is affected by papain.

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STUDIES OF CHRONIC INTOXICATIONS ON ALBINO RATS

V. ARSENIC TRIOXID

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The administration of arsenic was undertaken largely as a test of the availability of this type of experiments for chronic intoxications. To make this test severe, the doses were chosen very small; much smaller even than the quantities that are

TABLE 1

Mean dosage of arsenic trioxid, and duration of experiments

CONCENTRATION OF ARSENIC PER KILOGRAM OF FOOD	EXPERIMENT NUMBER	NUMBER OF ANIMALS IN EXPERIMENT	DURATION	MEAN DOSAGE OF ARSENIC TRIOXID PER KILOGRAM OF RAT PER DAY
mgm.			weeks	mgm.
0.001	54	6	24	0.0000475(0.00004 –0.00006)
0.001	120	3	9	0.000057(0.000048–0.000065)
0.003	53	4	14	0.00017(0.00012 –0.0002)
0.005	73	6	20	0.00032(0.00026 –0.00057)
0.01	55	6	24	0.0005(0.00039 –0.00067)
0.03*	5327	2	11	0.0015(0.0011 –0.0022)
0.05†	5492	2	19	0.0024(0.0013 –0.0031)
0.1‡	5597	2	19	0.0049(0.0027 –0.0082)

* After fourteen weeks of 0.003 (as experiment 53).
† After fourteen weeks of 0.001 (as experiment 54).
‡ After fourteen weeks of 0.01 (as experiment 55).

generally used as "tonics" in man or in animals. Contrary to expectations, the results did not show any increase in growth or appetite, but distinct, though moderate interference.

Dosage. This is shown in table 1. The weekly variations can be gathered from the chart of food-consumption. The

concentrations in the food ranged from 1:1,000,000,000 to 1:10,000,000; the mean daily dosage from 0.00004 mgm. to 0.0082 mgm. of As_2O_3 per kilogram of rat-weight, for nine to twenty-four weeks. The daily dosage would equal $\frac{1}{25,000}$ to $\frac{1}{125}$ grain for a man; i.e., $\frac{1}{800}$ to $\frac{1}{4}$ of the U. S. P. *single* therapeutic dose of $\frac{1}{30}$ grain.

It would not be safe to assume *a priori* that such doses would necessarily be harmless, when continued over long periods of time. According to the data given by Brouardel (page 33) for the Manchester epidemic of arsenic poisoning from beer (1900),
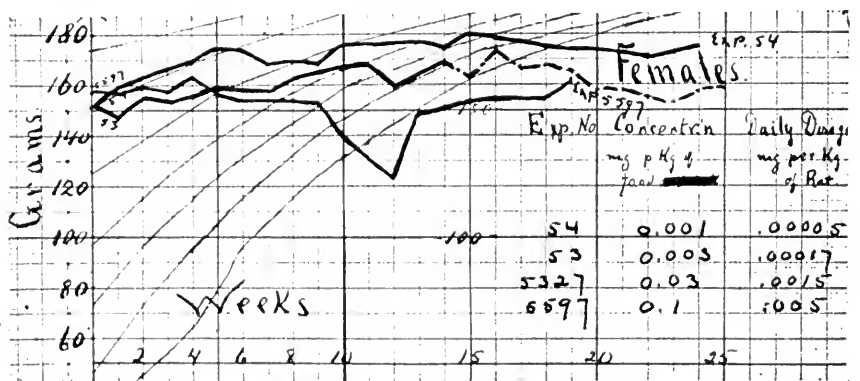
FIG. 1. EFFECTS OF ARSENIC ON THE GROWTH OF RATS

The heavy lines represent the growth of the arsenic rats; the light lines represent standard growth curves.

The broken lines represent Experiment 5327, as a continuation of Experiment 53.

toxic symptoms developed in individuals some of whom had probably consumed only from 0.13 to 4 mgm. of arsenic per day; i.e., $\frac{1}{240}$ to $\frac{1}{15}$ grain, or $\frac{1}{4}$ to 2 times the U. S. P. single dose.

On the other side there is, of course, the example of the Styrian arsenic eaters, who start with about 3 mgm. per day and increase gradually to 30 mgm. or more per day (Brouardel, page 62). These contradictions are not explained, but may be concerned with differences in absorption.

Effects on growth. Four typical curves are reproduced in figure 1. They show that the arsenic-fed rats generally failed to gain weight; the interference being more marked with the larger doses than with the smaller dosage. The same conclusion results from the numerical data, especially from the last column of table 2. The "percentile difference" from the standard, per week, may be grouped as follows:

Dosage of 0.00005 to 0.0005 mgm. = +1.1 to −1.4, mean −0.5
Dosage of 0.0015 to 0.005 mgm. = −1.0 to −2.1, mean −1.7

In the control series, the percentile differences in the growth of unpoisoned animals ranges between +1.8 and −1.0; mean

TABLE 2

Effects of arsenic trioxid on growth

DOSAGE OF ARSENIC TRIOXID PER KILOGRAM OF RAT PER DAY	EXPERIMENT NUMBER	DURATION	OBSERVED WEIGHT	NORMAL WEIGHT	DIFFERENCE	DIFFERENCE OF NORMAL WEIGHT	DIFFERENCE PER WEEK
mgm.		*weeks*	*grams*	*grams*	*grams*	*per cent*	*per cent*
0.0000475	54	24	175	20S	−33	−15.S	−0.65
0.000057	120	9	1S5	212	−27	−12.7	−1.4
0.00017	53	14	16S	1S2	−14	−7.6	−0:54
0.00032	73	20	233	1SS	+45	+23.9	+1.1
0.0005	55	24	1S0	206	−26	−12.6	−0.5
0.0015	5327	11	15S	196	−3S	−19.0	−1.7
0.0024	5492	19	11S	200	−S2	−41.0	−2.1
0.0049	5597	19	163	2C4	−41	−20.0	−1.0

= 0.13. The means of all the series of poisons, so far worked up, in which growth was not affected, ranges from +0.68 to −0.13. Those in which there was definite but not fatal interference with growth ranged from −0.2 to −2. It is clear, therefore, that arsenic interfered with the growth, the degree of interference increasing with the dosage. This is also the case in experiments in which small and large doses were given successively to the same group of animals (numbers 54 and 5492; 53 and 5327; 55 and 5597).

In one series (experiment 73) there was apparent stimulation of growth. In this series, however, there was a 50 per cent mortality, so that the ultimate growth curve is really that of the

extraordinarily vigorous survivors. It is therefore atypical and should be rejected; but as it does not alter the means materially, it has been included.

Effects on food consumption. This varies greatly in the different experiments, without much regard to the dosage, as may be seen from figure 2 and table 3. The mean percentile difference of the entire series is 3 per cent below standard. This is distinctly below the normal range (-0.08 to $+19$ per cent).

Comparison of the food and growth data, in table 3, shows that these always vary in the same direction, although they are not

TABLE 3

Food consumption

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	GROWTH, MEAN DIFFERENCE FROM NORMAL STANDARD PER CENT PER WEEK	FOOD CONSUMPTION MEAN DIFFERENCE FROM NORMAL STANDARD	
			Grams per rat per day	Per cent
	weeks			
54	24	-0.65	-0.9	-9.0
120	9	-1.4	-1.0	-10.0
53	14	-0.54	$+0.15$	$+1.6$
73	20	$+1.1$	$+2.2$	$+26.0$
55	24	-0.5	-0.2	-2.0
5327	11	-1.7	-0.2	-2.0
5492	19	-2.1	-0.4	-4.0
5587	19	-1.0	-1.4	-15.0

always strictly proportional; and the check in appetite is not quite as great as with most other measurers that cause an equal loss of weight.

Figure 2 shows that the interference with appetite increases materially with the duration of the arsenic feeding; i.e., the effect is not only continuous but progressive. This is not true of the effect on growth, for the growth line is nearly horizontal (fig. 1).

From these facts, it appears that factors other than loss of appetite must be concerned in the loss of weight.

Mortality. This is shown in table 4. With the dosage of 0.00005 to 0.0003, five deaths occurred for nineteen animals, i.e., 26 per cent. With doses of 0.0005 to 0.005, two deaths
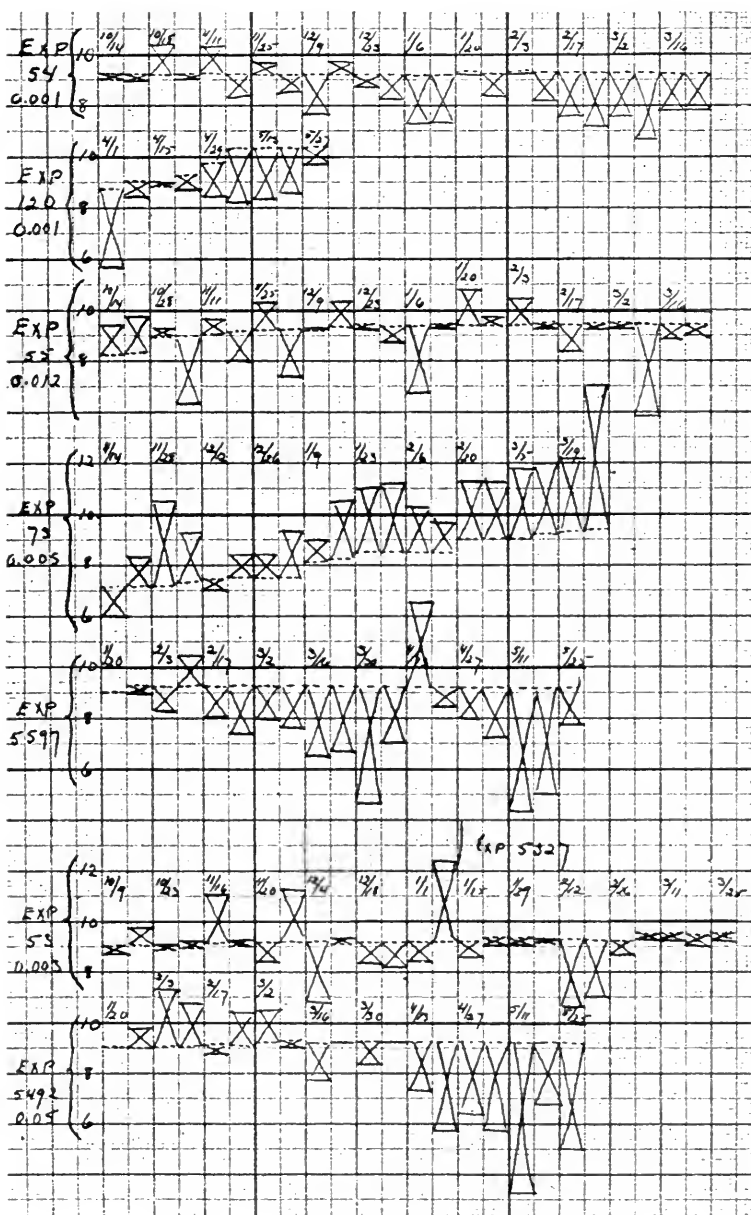
Fig. 2. Arsenic on Food Consumption

The numbers to the left represent grams of food consumed daily per rat. The numbers above each experiment are the dates of the observations. The dotted horizontal lines represent the standard food consumption; and the solid horizontal lines joined to the dotted lines by crossed lines represent the actual food consumption.

47

occurred for twelve animals, i.e., 17 per cent. The mortality is not high in either group, and is lower for the larger doses. This means that it must have been accidental, and not due directly to the drug.

Pathologic lesions. The tissue of the animals were examined by Dr. M. L. Richardson, who will report on them in a separate paper. He informs me, however, that there were no gross changes. Microscopically, there was slight cloudy swelling of the kidney and liver cells, and some congestion of the spleen. These were not marked. They tended to increase with the duration of the feeding, and showed but little relation to the daily dosage, i.e., they were predominantly cumulative.

TABLE 4

Mortality

EXPERIMENT NUMBER	NUMBER OF ANIMALS	WEEKS O˙ FATALITIES	TOTAL DURATION OF FEEDING WITH ARSENIC TRIOXID	FATALITIES˙
				per cent
54	6	8	24	16
120	3	0	9	0
53	4	1, 14	14	50
73	6	9, 9, 20	20	50
55	6	10, 11	24	33
5327	2	0	11	0
5492	2	0	19	0
5597	2	0	19	0

CONCLUSIONS

Arsenic trioxid, when administered to rats in their food over long periods (nine to twenty-four weeks) produces distinct retardation of growth and checking of appetite, in surprisingly small doses; i.e., 0.00005 to 0.0005 mgm. per kilogram of body weight; and more marked loss of weight with doses of 0.0015 to 0.005 mgm. per kilogram.

The loss of appetite is not parallel to the interference with growth, but is cumulative, i.e., it increases markedly with the duration of the feeding, whereas the check of growth is more

uniform throughout. The two actions are therefore probably fundamentally distinct, although they reinforce each other.

There is practically no mortality attributable to the drug.

The *daily* dosage in these experiment, when calculated on the average human weight, would amount to only $\frac{1}{800}$ to $\frac{1}{4}$ of the U. S. P. *single* therapeutic dose of 2 mgm. or $\frac{1}{30}$ grain. However, it is comparable with the dosage in the Manchester arsenic epidemic. The duration is doubtless an important factor. The results emphasize the need of surveillance of the weight, as well as of the urine, of patients subjected to arsenic administration for long periods.

The results contrast with the gain of weight that is popularly supposed to be produced in man and animals by small doses of arsenic. Perhaps this occurs only with larger, nephritic doses; and is not due to increase of flesh or fat, but to edema? This, of course, is not a conclusion, but a mere suggestion.

The results contrast also with the apparent harmlessness of arsenic to "arsenic-eaters." It is evidently unsafe to rely on the acquisition of such tolerance, which seems to occur only under special conditions, which are not understood.

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ON THE CHEMICAL COMPOSITION AND PHYSIO-LOGICAL CHARACTERISTICS OF BRAIN CEPHALIN

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Cephalin was isolated by Thudichum (1) and its hemostatic properties were discovered by Howell (2). The practical and clinical applications of this important phosphatide was demonstrated first by Hirschfelder (3) and later by Hess (4), Cecil (5) and others. Waksman (6) found that cephalin did not differ materially in thromboplastic activity, whether obtained from brains of cattle, hogs and sheep. It has also been pointed out by this author that the rate of deterioration in samples of cephalin is greatest shortly after preparation.

This investigation was carried out for the purpose of studying the characteristics of brain cephalin both from the physiological and chemical standpoint. The raw material suitable for the manufacture of cephalin for hemostatic purposes comprises brain and spinal cord from cattle, sheep and hogs. Experience has shown that brain substance alone yields a more active cephalin than spinal cord or a mixture of brain and spinal cord, and that cattle brains are best suited for the purpose and the present work has therefore been confined to this material.

The relations between the chemical and hemostatic properties of cephalin were determined on samples of varying degrees of purity. The usual methods of purification were followed. Fresh cattle brains still retaining their animal heat were carefully trimmed, washed free from blood, finely minced, and desiccated in vacuo (35°–40°C.). The dry material was ground coarsely and treated in Soxhlet extractors first with acetone and then with

ether. The acetone soluble fraction yielded a light yellow substance possessing a pronounced and characteristic odor. On exposure to the air it soon turns brownish in color. It is readily soluble in ether, but does not emulsify with water or isotonic salt solution and is devoid of blood clotting properties. This extract has a low ash, phosphorus and nitrogen content (see tabulation 1A). The ether extract was poured into three volumes of dehydrated ethyl alcohol and the mixture allowed to stand twenty-four hours in the refrigerator. The precipitated cephalin was centrifuged and without further drying redissolved in ether (approximately nine volumes). A white curdy precipitate which formed over night was separated by centrifuging, washed with ether and dried. It forms a cream colored, odorless, scaly substance which is readily reduced to powder form (tabulation 1B). The clear ether solution was concentrated to about half its volume and reprecipitated with excess of dehydrated ethyl alcohol. This process was repeated twice, giving the cephalin four alcohol precipitations in all. The alcohol washings were combined and evaporated to dryness at low temperature and reduced pressure. The tough amber-colored substance thus obtained is rich in lecithin, but contains among other things some cephalin to which its slight clotting power is due (tabulation 1C). The alcohol-insoluble portion which represents the purified cephalin was washed with dehydrated alcohol and dried in vacuo. This dried product is a light, orange-colored, friable substance, forming an almost clear solution in water and very active physiologically showing a clotting power of 1:1000 in thirty seconds (tabulation 1D). On standing it becomes darker in color and begins to lose some strength within two or three weeks after it is made. This confirms Waksman's findings (6).

Cephalin was also prepared from petroleum ether extracts of desiccated brain. After evaporation of the solvent the crude phosphatides appeared as light, amber-colored, transparent masses which soon became opaque on exposure to the air (for analytical data see tabulation 2A). This mass was cut into small pieces and washed three times with acetone until most of the fatty substances, cholesterol and other inert material were

removed. The light yellow, friable mass was next treated with an equal number of ethyl alcohol washings to remove lecithin, etc. The alcohol-insoluble portion constituting the cephalin was dissolved in ether and allowed to stand until the white, semi-colloidal precipitate had flocculated and settled. This sediment was washed separately with ether, centrifuged and dried (tabulation 2B). The cephalin solution was evaporated to dryness in vacuo. The yield of cephalin from the crude phosphatides is approximately 35 per cent. The amber-colored, purified cephalin is of waxy consistency and retains its activity quite well when stored in dark brown, well stoppered bottles. The clotting power of this preparation was 1:1000 in forty seconds (tabulation 2C). This sample is not pure cephalin; it was chosen as the control on account of its relatively high thromboplastic activity and its excellent keeping qualities.

During the last few years more than a dozen batches of cephalin have been prepared, on a large scale, according to this method. These preparations maintain their physiological activity practically unaltered for nine to fifteen months. The time limit for commercial cephalin of one year, as suggested by Hanzlik and Weidenthal (7), seems fair both to the consumer and the manufacturer.

When such cephalin is purified further by successive alcohol precipitations the results are quite similar to those obtained by the first described method. Sample 2D was precipitated twice and sample 2F three times with dehydrated ethyl alcohol. The yields however are small since cephalin itself is soluble to some extent in dehydrated alcohol. This would indicate that we have reached the limit of the ether-alcohol purification and are dealing with fairly pure specimens of cephalin.

Preparations were also made directly from fresh brain tissue. A sample prepared according to the method outlined in New and Nonofficial Remedies (8), was of a pale yellow color and showed a clotting power on oxalated blood of 1:1000 in one hundred seconds. A more active preparation was obtained by dehydrating and extracting the fresh brain substance with acetone. After three successive macerations the acetone was

removed by suction and alcohol washing and the residue macerated three times with alcohol. The residual brain substance was then extracted with ether in a Soxhlet extractor. The ether solution, after standing and removal of the heavy white precipitate, was evaporated to dryness. The cream colored substance curdled oxalated blood in one-half the time required by the sample given in the New and Nonofficial Remedies. It may be concluded therefore that preparations made from fresh brain tissue are lighter in color than those obtained from desiccated brain but not superior in hemostatic properties.

The thromboplastic activity of all samples described in this paper was tested according to the oxalated blood plasma method which furnish a reliable and convenient means of identifying good cephalin preparations. Cephalin emulsions which curdle oxalated blood plasma in from one-half to one and one-half minutes give excellent clinical results in the hands of experienced operators. The method is carried out in this laboratory. as follows: 1 cc. of a 5 per cent solution of cephalin in ether is added to 25 cc. of cold isotonic sodium chloride solution and the mixture shaken until a uniform suspension results. After warming to body temperature 5 cc. of this suspension, are mixed in a large test tube with an equal volume of blood serum. Then 10 cc. of oxalated blood plasma are added and the liquids mixed by inverting the test tube twice. The mixture is rapidly transferred to a shallow porcelain dish and the clotting time noted. This should not exceed two minutes. The endpoint when a solid clot is formed, is distinct and readily observed. All solutions should be freshly prepared and the temperature of the liquids and the porcelain dishes maintained at 38°C. by means of a constant temperature bath.

In preparing and standardizing biological products it is important to eliminate individual variations as far as possible. The number of animals represented in a lot of cephalin often runs into the hundreds. Beef serum and plasma were employed for testing purposes because both are obtainable, free from contaminations, in the daily routine of the abattoir. The serum is centrifuged and is a composite from a large number of animals.

The blood is received directly from the bleeding animal in wide mouth bottles of 100 cc. capacity, each containing 10 cc. of a 1 per cent sodium oxalate solution in distilled water. Twelve to fifteen bottles from as many animals are generally collected. The bottles are stoppered immediately and shaken to insure complete mixing of the blood and oxalate solution. After removal to the laboratory the contents of the bottles are inspected, mixed and prepared for test. If any specimen shows clot or ropy plasma it is discarded. This procedure insures a representative plasma which furnishes concordant results. After standing for a few hours, oxalated blood does not clot as promptly as when freshly prepared. The importance therefore of running control samples of known strength alternately with the unknowns should be borne in mind.

In the tabulation will be found the yield and clotting power of the various preparations, also their loss in moisture and ash contents. Phosphorus was determined on 0.5-gram samples by adding 2 cc. of sulphuric acid and several small portions of nitric acid, until oxidation was complete. The clear liquid was diluted with water and the phosphorus determined by the official volumetric method calculating to phosphorus pentoxide. Total nitrogen determinations were made according to the official Gunning-Kjedahl-Arnold method. The sodium and potassium were determined on the ash, which was obtained by incinerating at a temperature below dull red heat. The iodine value was determined according to the Wijs method.

The most striking feature of the physiologically active cephalin is the high ash content, consisting of potassium and sodium salts of phosphoric acid. The ash is water soluble and slightly alkaline in reaction in contrast to the ash from the crude phosphatides and from the lecithin fractions, which are distinctly acid. This is obviously because cephalin contains much more sodium and potassium in proportion to the phosphoric acid than the other two substances. It should be remembered that the lecithin fractions mentioned here are impure lecithin containing among other things some cephalin. The ash content of pure lecithin is undoubtedly much lower than our figures

Chemical and physiological properties of the acetone, ether and petroleum ether soluble portions of cattle brain

	YIELD FROM DESICCATED BRAIN TISSUE per cent	YIELD FROM ORIGINAL FRESH BRAIN TISSUE per cent	MOISTURE per cent	ASH per cent	K₂O per cent	Na₂O per cent	P₂O₅ per cent	TOTAL NITROGEN per cent	IODINE VALUE	CLOTTING POWER ON OXALATED BLOOD	CONDITION OF EMULSION	CONDITION OF CLOT	REMARKS
1A. Acetone extract from desiccated cattle brain	22.20	4.75	2.40	1.25			2.45	1.62	110.6	No clotting power	Does not emulsify		
1B. Precipitate formed in ether solution on standing	2.00	0.43	1.70	6.55			3.70	2.60		No clotting power	Swells up in water but only slightly soluble		These three fractions constitute the ether extract of desiccated cattle brain previously extracted with acetone
1C. Alcohol soluble portion of ether extract. "Impure lecithin"	12.10	2.58	1.00	5.90	0.70	0.57	7.05	2.33	77.0	Slight clotting power	Milky emulsion. Insoluble residue	Very soft	
1D. Alcohol insoluble portion of ether extract. "Purified cephalin"	8.90	1.90	4.60	14.95	3.65	1.88	8.51	2.18	42.3	1:1000 in 30 seconds	Straw-colored almost clear solution	Firm	
2A. Petroleum ether extract from desiccated cattle brains. "Crude phosphatides"	45.00	9.63	2.80	6.63	1.30	0.57	4.92	1.67	91.5	1:1000 in 150 seconds	Milky emulsion. Much insoluble material	Soft	

Description										No clotting power	Swells up in water	Consistency	Notes
2B. Ether insoluble residue after acetone and alcohol washing of crude phosphatides	2.57	0.55	1.50	5.47	2.01	0.43	3.17	2.66		No clotting power	Swells up in water—mostly insoluble	Firm	Free fatty acids as mgm. of KOH per gram sample 47.4. Refractive index at 50°C. 1.4886
2C. Cephalin made from crude phosphatides. "Control cephalin"	15.75	3.37	3.10	9.50	1.78	0.98	7.10	1.97	80.4	1:1000 in 40 seconds	Milky emulsion	Firm	
2D. Control cephalin 2C. Purified further by two ether-alcohol precipitations. Alcohol insoluble portion. "Purified cephalin"	6.30	1.35	1.50	15.06	3.63	2.18	8.76	2.02	59.6	1:1000 in 35 seconds	Straw-colored, opaque solution	Firm	
2E. Alcohol soluble fraction from 2D	9.45	2.02	1.20	3.70	0.52	0.46	5.54	1.68	101.4	Unsatisfactory clot in about 3 minutes	Imperfect emulsion, considerable insoluble material	Very soft	
2F. Control cephalin purified by three ether-alcohol precipitations. "Purified cephalin"	5.50	1.18	2.50	15.43	3.77	2.00	8.70	1.80	46.8	1:1000 in 30 seconds	Straw-colored, almost clear solution	Firm	

	YIELD FROM DESICCATED BRAIN TISSUE	YIELD FROM ORIGINAL FRESH BRAIN TISSUE	MOISTURE	ASH	K_2O	Na_2O	P_2O_5	TOTAL NITROGEN	IODINE VALUE	CLOTTING POWER ON OXALATED BLOOD	CONDITION OF EMULSION	CONDITION OF CLOT	REMARKS
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent					
2G. Control cephalin emulsified with water and precipitated with N/1 HCl	*	*	1.30	0.31			5.27	1.17	No definite iodine value could be established	No clotting power	Does not emulsify		Free fatty acids as mgm. of KOH per gram sample 79.4. Refractive index at 650°C. 1.4788
2H. Neutralized (NaOH) acid water filtrate from 2G. Desiccated	*	*					2.54	0.70					Consists chiefly of sodium and potassium chloride and phosphate
2I. Control cephalin twice emulsified with water and twice salted out with NaCl. Alcohol insoluble portion. "Purified cephalin"	4.00	0.86	1.46	9.90	0.22	2.65	7.40	1.61		1:1000 in 35 seconds	Straw-colored, slightly turbid solution	Firm	No chlorides present
2K. Alcohol soluble portion of 2I	*	*	None	3.67	0.14	0.87	5.28	1.48		No clotting power	Does not emulsify		No chlorides present

* Not determined.

show. Chlorides and sulphates are absent and the ash did not contain any calcium and magnesium as claimed by Thudichum (1). A slight trace of iron was present.

Another interesting point is the iodine absorption values of the various preparations. The two best refined and most active samples of cephalin in this series (1D and 2F) have iodine numbers of 42.3 and 46.8 respectively. The sample next in activity and purity (2D) shows 59.6, and the still more impure control cephalin 80.4. The iodine value of the acetone extract (cholesterol etc.) (1A) is 110.6 and that of the alcohol soluble fraction (lecithin, etc.) (1C) 77.0. It is evident that the iodine value of pure cephalin must be quite low.

The effect caused by the removal of the sodium and potassium from cephalin was next studied. The method originally outlined by Thudichum (1) for producing ash free cephalin comprises the emulsification in water of the purified phosphatide and subsequent precipitation with dilute hydrochloric acid. Samples of the control cephalin were emulsified with water (1:100). This was accomplished by mixing a 10 per cent ether solution of cephalin with the required amount of cold distilled water, shaking vigorously and permitting the ether to evaporate by immersing the container in warm water at 45–50°C. After standing for some time (varying from a few hours to two days) just enough N/1 HCL was added to produce separation. The flocculent precipitate was collected, taken up in ether and filtered. When these ether solutions were poured into dehydrated alcohol in order to precipitate the cephalin, it was found that over 90 per cent of this substance had become alcohol soluble. The alcohol-insoluble fractions were dark in color and showed some physiological activities (1:1000 in sixty-five to seventy seconds). A second emulsification and acidulation rendered this cephalin alcohol soluble. The alcohol soluble substance after drying was of sticky consistency, reddish brown in color and physiologically inert. The emulsifying properties were likewise lost. By consulting the tabulation (experiment 2G) it will be seen that while practically all the ash was removed by the treatment, only a relatively small percentage of the total

phosphorus was lost. The increase in the free fatty acids content of this sample over that of the original control cephalin is also very significant. The refractive indexes of the two samples also differ. The iodine value of the control cephalin was 80.4. The cephalin preciptated with dilute hydrochloric acid did not yield concordant results even from identical samples. Duplicate determinations would vary from 77.6 to 86.8 showing that in this case it is probably not a question of simple addition, but of addition and substitution. It is evident therefore that cephalin at least in part is a sodium and potassium soap and that the removal of the two bases materially alters the physical, chemical and physiological characteristics of the phosphatide. Test for chlorine was negative, showing that we are not dealing with cephalin hydrochloride as stated by Thudichum. This fact has been pointed out by Levene and West (9), and others.

The acidulated water filtrates from the cephalin precipitations were neutralized with sodium hydroxide and evaporated to dryness. The dry crystalline residue consisted principally of sodium and potassium chloride but contained also phosphates to the extent of 2.54 per cent P_2O_5 and 0.7 per cent of nitrogen (tabulation 2H). Thudichum reported the presence of copper in this liquid. Our samples were handled in glass and porcelain only and did not show any copper.

In order to determine if the bases could be leached out simply by water extraction, samples of the control cephalin were first emulsified in water and then salted out by means of finely powdered sodium chloride to saturation. The cephalin was taken up in ether and again emulsified with water. The second emulsification was complete and even more readily accomplished than the first one. After the second salting out the cephalin was dissolved in ether, precipitated with dehydrated alcohol, centrifuged and redissolved in ether. The ether solution was very turbid. On centrifuging a heavy white precipitate consisting chiefly of sodium chloride separated out, leaving a clear, supernatant liquid. After evaporation of the ether this cephalin was transparent, of dark amber color and showed a clotting power of 1:1000 in thirty-five seconds. Analytical data of both the

alcohol soluble and insoluble fractions are given in the table (experiments 2J and 2K). None of these samples contained chlorides, showing that all the salt used for precipitation purposes was properly removed. It will be seen that this cephalin resembles other purified cephalins in physiological behavior, but differs chemically in the loss of considerable potassium soap. The potassium compound evidently is split to a large extent by the water and salting out treatment, while the sodium soap apparently is not hydrolyzed in this process.

The highly purified samples of cephalin prepared in this laboratory made straw colored aqueous solutions. Some of these solutions were almost clear other opalescent. Less pure cephalin gave milky emulsions containing some suspended matter. The alcohol-soluble (lecithin) fractions behaved in a like manner. The acetone-soluble portions (cholesterols, etc.) did not emulsify at all. Cephalin seems to be the only brain phosphatide which is readily soluble in water.

Cephalin solutions are very sensitive to even slight degrees of acidity. An extract of cattle brain in physiologic sodium chloride solutions prepared according to the method of Hess (4) and used for hemostatic purposes under the name of thromboplastin solution is slightly acid in reaction when freshly prepared. It looses very materially in activity in the course of a few months and eventually becomes inert unless the acidity is carefully neutralized by means of sodium hydroxide. The neutralized samples retain their activity much longer.

The white semi-colloidal precipitate occurring in all ether solutions of cephalin contain more nitrogen and less phosphorus than the phosphatides. The relatively large amounts of sodium and potassium indicate however that this substance is a derivative of cephalin. It is probably formed by progressive hydrolysis of cephalin in the presence of small amounts of moisture and acid occurring in the liquids with which cephalin comes in contact during the various purification treatments. Freshly and properly prepared cephalin forms a clear solution in ether. In the course of time the liquid invariably separates out a white precipitate. After this reaction has taken place the solution

remains clear and apparently unchanged for years, provided it is tightly sealed. A number of ether solutions of cephalin have been under observation in this laboratory over a period of three and one-half years. They maintain their physiological activity far better than the dry cephalin kept in stoppered bottles.

The reason that less pure cephalin in the dry form keeps better than the highly purified substance is probably because the former contains fatty substances which protect the cephalin from deterioration. Further work on this problem is in progress.

The writer wishes to acknowledge his indebtedness to Miss Mary Hull, Miss Mable Smith and Mr. Joseph Brunn for assistance in carrying out this work.

CONCLUSIONS

Brain cephalin contains considerable potassium and sodium. These two bases occur as soaps in combination with some of the fatty acids constituting the phosphatide. Both the solubility of cephalin in water and its thromboplastic properties depend on the presence of these soaps.

Potassium, sodium and a faint trace of iron were the only inorganic bases found in the samples of cephalin examined.

In the dry form moderately pure cephalin is more stable than the highly purified substance. In ether solution cephalin retains its physiological activity practically unchanged for several years.

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THE PHARMACOLOGY OF CHELIDONIN, THE BEN-ZYLISOQUINOLINE ALKALOID OF CHELIDO-NIUM (CELANDINE OR TETTERWORT) AND STYLOPHORUM

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CONTENTS

I. INTRODUCTION

The results of previous studies with chelidonin by H. H. Meyer (1) on general and systemic actions and by me on smooth muscle (2) indicate that this drug possesses a distinct field of pharmacological and therapeutical usefulness. This was considered sufficiently important to justify a further and more extensive pharmacological study of this alkaloid. Some errone-

ous impressions concerning the actions of this alkaloid were corrected in a recent report (3). It is the object of this paper to present a comprehensive survey of its physical and chemical properties and pharmacological actions.

The following brief résumé of the chemical and physical properties of chelidonin was thought desirable because its literature is fragmentary, being scattered through several works on botany, pharmacognosy, toxicology and pharmacology. Important studies on chelidonin formerly engaged the attention of several American pharmacists and chemists. Some of these studies are unusually complete, but appear to have been generally overlooked. With the elimination of the crude drug celandine or tetterwort from the materia medica its alkaloid, chelidonin, has been practically forgotten. However, it will be seen from the results of this paper that its actions are important and merit at least as great a recognition as has been accorded to papaverin, the advantages, probably being on the side of chelidonin. This will be discussed later in the text.

Abstract of literature

The word "chelidonium" is derived from the Greek, "chelidon," a swallow, since the plant blooms during the entire period of the swallow's sojourn, presumably in European countries. The most important alkaloid of chelidonium (also stylophorum) is chelidonin, which occurs to the extent of about 0.03 per cent in the roots, the remaining portions of the plant containing only traces. Other alkaloids of chelidonium described by E. Schmidt (4) and others are homochelidonin, chelerythrine, protopin, and also cheldoninic acid. A description of these and their pharmacological actions may be found in the paper by H. H. Meyer (1). The following brief summary will be limited to chelidonin.

Properties. According to Schlotterbeck and Watkins (1901) (5) chelidonin was first isolated in an impure state from Chelidonium majus by Godefroy in 1824. The alkaloid was later further purified and described by Probst, Polex and Reuling and Eykman. Schmidt and Selle isolated the alkaloid from

stylophorum. Selle thought chelidonin was closely related to morphin. The constitution was left indecisive by the work of Wintgen (6) and Henschke (7), although the empirical formula, established $(C_{20}H_{19}NO_5.H_2O)$ by these earlier workers agrees with that of later workers. In 1885 Professor John Uri Lloyd found a white, crystalline alkaloid in Stylophorum diphyllum (Yellow Poppy) which was identified as chelidonin by Professor Eykman of Tokio, Japan.[1] In 1903 Schlotterbeck and Watkins (8) showed that chelidonin contains benzyl groups, but no aldehyde, ketone or methoxyl groups. In 1905, these authors (9) showed further that this alkaloid contains no phenanthrene and, therefore, is not a morphin. Besides a study of chemical reactions it was also established by these authors that chelidonin is the principal alkaloid of Chelidonium majus and Stylophorum diphyllum. The following structural formula has been recently proposed by J. Gadamer (10).
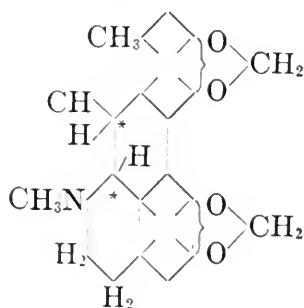
* = asymmetric carbon atoms.

Gadamer confirms the absence of methoxyl groups previously reported by Schlotterbeck and Watkins, and describes the following additional properties: Chelidonin is a tertiary base, optically active $[\alpha]_D^{20} = +115.4°$ in 96 per cent alcohol; contains a methyl-imid group and an alcoholic group. Accordingly the lesser toxicity of chelidonin as compared with papaverin may be accounted for by the absence of methoxy groups, two of which are present in papaverin.

[1] Private communication.

Color reactions (11). The following color reactions for chelidonin have been described:

With guaiacol and H_2SO_4 (sp. g. 1.84).....Bright carmine; persists
With thymol and H_2SO_4.................Rose color lasting 24 hours, later purple
With oil of cloves and H_2SO_4 ....Purple
With naphthol and H_2SO_4................Dark green streaks
B-naphthol.............................Brown, passing to violet
With pyrocatechin......................Rose color, to violet
With $\begin{cases} \text{hydroquinone} \\ \text{resorcin} \\ \text{phloroglucin} \end{cases}$ ....Yellow
With pyrogallol........................Red to yellow
With galoltannic acid...................Yellow to green

Additional color reactions are given by Gadamer (12), but will not be described here, since this information is readily accessible to anyone sufficiently interested.

Medicinal uses. These have been catalogued from various sources (13). The entire plant, chelidonium, including the juice, has been used as diuretic, purgative, stimulant, alterative, diaphoretic, vulnerary, drastic hydragogue, in hepatic disease, leprosy and cancer. Alcoholic extracts of the plant have been recommended for scrofula, skin eruptions, and amennorrhea. The milky juice of the plant is almost universally quoted as a caustic for removing warts, corns, in eczema, etc. The alkaloid, chelidonin, has been used for relief of gastric and intestinal pains. The dosage used by Ribbing and Rumpf (1) ranged from 0.1 to 0.2 gram for gastralgias accompanying ulcer and malignancy. The advantages over opium were claimed by the older authors to be; no constipation, drowsiness or stupor and no unpleasant effects whatever. Dragendorff states that it has been used as an analgesic in snake-bite. According to Gadamer (12) chelidonin is not very poisonous, but the milky juice from the plant is irritating to the skin and mucosae, causing wheal formation and inflammatory reactions.

Pharmacology. The uses described above are largely empirical and preceded the studies on general and systemic actions by H. H. Meyer (1) in 1892; those by Worth Hale in 1908 (15; 16)

on the cord and perfused frog's heart and those by myself in 1915 (2) on smooth muscle in various regions. The results of Meyer have been adequately described in previous papers and need not be reviewed in full. Only one item requires special attention, and that is the mechanism of cardiac slowing. Meyer attributed this to peripheral vagus stimulation by chelidonin. This seems inconsistent with the results of Hale on atropinized frog hearts, which were also slowed by chelidonin. However, in the experiments of Hale, the functional activity of the vagus was not tested, and observations were not made on hearts of other species. Therefore, this proposition was tested out in the experiments to be reported in this paper. It was found that the functional activity of the vagus nerves was not necessary for the slowing. This would be expected from what is known about the related alkaloid, papaverin, which also depresses cardiac muscle independently of nerve connections. In fact, the functional activities of all muscles (cardiac, skeletal and smooth) are depressed by chelidonin. Meyer showed that frog's skeletal muscle is depressed by weak and paralyzed by strong concentrations and undergoes degenerative changes. Smooth muscle of the intestine, stomach, esophagus, uterus and blood vessels was shown by me to be depressed also. The evidences on bronchial muscle were incomplete, and the smooth muscle of some other important regions had not been studied at all. Because of this and other discrepancies, and the doubt regarding the cause of cardiac slowing mentioned above, it seemed worth while to reinvestigate certain of the actions of chelidonin and to extend the studies in several directions. It should be mentioned also that Hale found the spinal cord to be depressed by chelidonin as indicated by the diminished response of skeletal muscular contractions on electrical stimulation of the lumbar cord in injected frogs.

Chelidonin sulphate obtained from two different sources was used in this study. The specimen of German origin was obtained from Merck and that of American origin from Prof. John Uri Lloyd, who used Chelidonium majus for one specimen, and Stylophorum diphyllum for another specimen. The alkaloids

from those two plants were nearly identical and free from other alkaloids found in these plants. I am greatly indebted to Professor Lloyd for his generous supplies of the alkaloid. In a previous communication it was stated that only the sulphate and phosphate are soluble, the hydrochlorid being very insoluble. Professor Lloyd states that in his experience the acetate is the most soluble salt, the picrate being the most insoluble. Only the sulphates were used in my work. The results obtained may now be described.

II. PHARMACOLOGICAL ACTIONS

1. Fatal and therapeutic dosage

The minimal fatal dosage of chelidonin sulphate was determined by subcutaneous injection in white rats, guinea-pigs, mice, frogs, rabbits, cats and dogs. The results are summarized in table 1. According to this the minimal fatal dose is about 0.3 gram per kilo for the different species studied, except cats.

Data from the literature indicate that papaverin is more than twice as toxic as chelidonin by hypodermic injection. This is shown by the summary in table 2. Accordingly, the effective therapeutic dosage of chelidonin may be higher. Outside of the therapeutic dosage of $1\frac{1}{2}$ to 3 grains used successfully by Ribbing and Rumpf no data on chelidonin in clinical practice are available. I have administered 3 grains of the sulphate by mouth to three different individuals without any disturbing effects whatsoever, except in one individual who claimed to have experienced a loose stool and desire to defecate. Diarrhoeal effects in rabbits have been occasionally observed in the various experiments performed.

The fatal dose by intravenous injection was not accurately determined. It is much less than that by subcutaneous injection. A tabulation of the responses to a wide range of dosage used in the different experiments indicates the following results: Out of nine dogs receiving a range of doses from 0.005 to 0.044 gram per kilo intravenously in 1 to 11 divided portions, respectively, not a single one survived, but a cat that received 0.025

gram per kilo in 3 divided portions was not killed. Meyer's experiments indicate the same tendency. That is, 2 of his cats, receiving 0.043 gram and 0.094 gram per kilo intravenously, were not killed, while a dog receiving 0.015 gram per kilo died. 0.3 gram per kilo subcutaneously never failed to kill members of the other species studied, but this quantity was not fatal to cats, confirming the tendency exhibited with intravenous administration.

TABLE 1

Minimum fatal dosage of chelidonin subcutaneously in different species

SPECIES AND NUMBER OF ANIMALS INJECTED.	RANGE OF DOSAGE USED	MINIMUM FATAL DOSE
	grams per kilogram	*grams per kilogram*
White rats (7).................................	0.065 to 0.4	0.3
Guinea-pigs (3)...............................	0.1 to 0.3	0.3
Mice (6)..	0.05 to 0.4	0.3
Frogs (3).......................................	0.1 to 0.3	0.2
Rabbits (5)....................................	0.006 to 0.3	0.26
Cats (4)..	0.05 to 0.3	>0.3
Dogs (3).......................................	0.2 to 0.4	0.3
Median..		0.3

TABLE 2

Comparison of chelidonin with papaverin (gram per kilogram)

DOSE	PAPAVERIN	CHELIDONIN
Fatal (subcutaneously)...............	0.128 (cat; dog)	0.3 (several species)
Lowest effective (intravenously).......	0.1 (rabbit)	0.00055 (dog; cat)
Narcotic (subcutaneously)............	0.06 (cat) 0.25 (rabbit)	0.1 (cat)
Therapeutic (by mouth for adult man)	0.03 to 0.08	0.1 to 0.3

Meyer's results also indicate that rabbits can survive higher doses than dogs. For instance, one animal survived 0.019 gram per kilo, but the other was killed by 0.065 gram per kilo intravenously. It seems certain, therefore, that cats are more resistant to chelidonin than rats, guinea-pigs, mice, frogs, rabbits, and dogs.

On the other hand, dogs appear to be more sensitive than cats and rabbits when the drug is injected intravenously. This

can not be attributed to the concentration of the drug injected, for this was uniform (5 per cent) in my experiments and different from that used by Meyer, whose results, nevertheless exhibited the same tendency as mine.   There was also no relation to the weight of the animals, or to the absolute dosage of the drug used.   However, the number of divided doses injected went parallel with the increase in dosage.   In turn the total dosage was more or less proportional to the level of blood pressure, or, in other words, the efficiency of the circulation.   The cardiac factor in this, of course, is of paramount importance.   The heart, being correspondingly reduced in efficiency and resistance with lower levels of blood pressure, was more susceptible to the depressant influences of the chelidonin and required, therefore, fewer doses to cause stoppage.   This may not adequately explain the species difference between cats and dogs, but, at least, indicates the importance of the circulation, especially the heart, in intravenous injections.   It may be that dog's heart is more susceptible to the injurious action of chelidonin than cat's heart.   This illustrates the need of caution in transferring results from one species to another, especially in the case of drugs like chelidonin intended for therapeutic purposes.   No data are as yet available for the susceptibility of the human heart to chelidonin, but if this should approach that of dog's heart, caution should not be overlooked in the routine administration of the drug. This applies also to the allies of chelidonin, namely, papaverin and benzyl benzoate, for, it must be remembered that all of these agents are efficient cardiac depressants.   The cardiac depressant effects may even make their appearance before the desired therapeutic relief from spasm of smooth muscle is obtained. This is illustrated in a recent paper by Mason and Pieck (17) on the antiperistaltic action of benzyl benzoate on the intestine and uterus of dogs.   The heart was injured (dilated or stopped) almost invariably before any effects on peristalsis could be demonstrated.   On the other hand, epinephrin under the same conditions inhibited intestinal peristalsis promptly without injury to the circulation.

Therapeutic dosage. The summary in table 2 indicates that the therapeutic dose of papaverin is about one-third to one-half of that of chelidonin. The toxicity of papaverin for animals is also seen to be greater (one-third). Narcotic effects or tendency to drowsiness in cats required a greater (1.6 times) dosage of chelidonin than papaverin, namely, 0.1 gram per kilo subcutaneously. Rabbits appear to be more resistant to chelidonin than other species. The minimal effective dose of chelidonin by intravenous injection for dogs in my experiments was 0.00055 gram per kilo equivalent to about $\frac{1}{2}$ grain for an adult man of 60 kilos. The definitely and markedly effective dosage in the majority of animals was from 0.0025 to 0.005 gram per kilo intravenously, or equivalent to about $2\frac{1}{2}$ to 5 grains for an adult. It seems, therefore, that the toxicity of chelidonin when given by mouth or subcutaneously to man would be rather low as compared with other alkaloids of the papaveraceae. The correct adult dosage for therapeutic purposes appears to be about 3 grains (0.2 gram).

2. *Symptoms*

These are similar in the different animals studied, namely, dog, cat, guinea-pig, rat, mouse, rabbit and frog. The effects of doses less than 0.3 gram per kilo subcutaneously are characterized by moderate depression, some narcosis, loss of equilibrium, moderate slowing of the pulse and either no change or some increase in rate of respiration. Dogs and cats vomited as a rule within 10 minutes after injection. The contrast with morphin action is particularly noticeable in cats. Chelidonin does not cause the marked excitation characteristic of morphin on cats. Observations were made on 3 cats injected with 0.05 gram, 0.083 gram on 0.3 gram per kilo subcutaneously. The following protocol illustrates the typical action on the cat that received 0.083 gram of chelidonin sulphate per kilo.

Protocol

Cat 1.2 kilos

	TIME	PULSE	RESPIRA-TION	REMARKS
Control		312	60	
Chelidonin	30 min.	264	54	Slight depression; no rest-
0.083 gram per	57 min.	252	76	lessness or excitation
kilogram	1 hr., 27 min.	240	84	Drowsy
	2 hrs., 45 min.	288	54	No restlessness; not depressed

The effects on the other two cats were similar, being somewhat more pronounced on the cat receiving 0.3 gram per kilo. Protocols of the remaining species will not be presented since they contain nothing unusual or different from this cat. Rodents, of course, did not vomit. White rats responded somewhat variably to the lower doses of chelidonin, as is the custom of these animals towards poisons in general. High doses produced chiefly depression. Doses of 0.2 to 0.3 gram per kilo did not cause the tail raising effect in rats as described by Straub for mice with morphin, and by Jensen and Rumry (21) with nicotin.

The symptomatic effects of chelidonin on lower animals here reported confirm in general those observed by Meyer and agree with those obtained with papaverin by several investigators.

The administration of 3 grains (0.2 gram) by mouth to three different human subjects produced no symptoms whatever in two. One man passed extra stools and expressed a greater desire to defecate the same night. Similar experiences have been reported in the literature with papaverin. Although the contrary, i.e., constipation, would be expected with agents which relax the plain muscle of the bowel, yet this same effect could conceivably produce a better evacuation by relaxation of the bowel in spastic or tonic contraction.

3. Circulatory effects

Studies on the intact circulation were made principally on dogs, observing the effects of intravenous injection of chelidonin on blood pressure, pulse rate, cardiac volume and peripheral

organ (kidney) volume. Observations were also made on the excised and atropinized hearts of frogs and turtles, and excised arteries.

The following methods were used. Dogs were previously anesthetized with ether, tracheotomized and a carotid artery was connected by means of cannula and tubing to a damped mercury manometer for recording the mean blood pressure in the usual way. A metal stopcock cannula was tied in the saphenous vein for making the injections. Using artificial respiration, the chest was opened for introduction of the cardio-plethysmograph to enclose the heart according to Y. Henderson. This was attached by means of rubber tubing to a finely adjusted tambour of moderate size, for recording changes in the volume of the heart. Air conduction was used in the system. The left kidney was used for recording changes in peripheral organ volume as an index of vascular changes in the periphery. The organ was placed into a convenient oncometer attached by rubber tubing and air conduction to a tambour which recorded the changes on the kymograph. All effects, i.e., changes in blood pressure, pulse rate, cardiac volume and kidney volume were simultaneously recorded on a slow moving kymograph. Some of the animals were curarized, using 0.6 cc. of 0.5 per cent curare (Buchet et Cie, Paris) extract per kilo, in order to elimi-nate the effects of struggling and tonicity of skeletal musculature. The activity of the different organs having reached a constant position or level as indicated by the tracings, the drug was now injected, using a range of different concentrations, and the effects noted.

The effects on the heart were further controlled by perfusion of excised hearts of frogs and turtles. This was done as follows:

The Straub method of perfusing the ventricle was used with frog hearts. A long narrow glass cannula was inserted through the aorta into the cavity of the ventricle and securely tied. The remaining vessels were then tied and the excised heart was transferred to a moist chamber supplied with a constant stream of oxygen, a part of which was conducted at the same time into the ventricular cannula, containing Ringer's solution. The

chelidonin in different concentrations in Ringer's solution was introduced directly into the cannula and washed out by means of suction and a fine pipette. A light lever attached to the apex of the ventricle by means of a fine wire clip and silk thread passing through an opening in the bottom of the chamber were used to record the contractions on a slow moving kymograph.

The hearts of turtles were perfused in the usual way by placing cannulas into a vein and into an aorta. The vein cannula was connected to two bulbs by tubing with a by-pass for convenient removal of the drug solution from one of the bulbs. The other bulb was used for plain Ringer. The remaining vessels were ligated, leaving the vagus nerves intact and perfusing the heart in situ while the contractions were recorded on a slow moving kymograph. For paralyzing the vagus endings 0.1 per cent solution of atropin sulphate was used with the frogs and turtles.

The results of the injection experiments on the 6 dogs that were used are presented in table 3. These indicate the responses to different doses of chelidonin at different levels of blood pressure in untreated and atropinized animals. Figure 1 illustrates typical effects of chelidonin on the mammalian circulation. Figure 2 illustrates the effects of chelidonin on an atropinized turtle heart.

The results obtained show that the intravenous injection of chelidonin causes invariably a prompt fall of blood pressure with slowing of the pulse, increase in cardiac volume (diastolic tendency) and a fall in peripheral organ (kidney) volume. That is, all the effects are in the same direction and, therefore, are of cardiac origin. The effects are roughly proportional to the dosage, being more pronounced with greater dosage, although the degree of response varied. It was not possible always to predict the degree of effect of a given dosage. For instance, in one animal (dog 18.5 kgm.) a single dose of 5 mgm. per kilo caused profound effects ending in cardiac paralysis, the initial blood pressure being at the level of 100 mm. In another animal (dog 9.0 kgm.) 10 doses, ranging from 0.55 to 11 mgm. per kilo, were necessary before a fatal effect was produced, the initial

TABLE 3

*Circulatory effects of chelidonin on dogs**

DOSE OF CHELIDONIN INTRAVENOUSLY	CHANGE IN PULSE RATE PER MINUTE	CHANGE IN BLOOD PRESSURE	CHANGE IN CARDIAC VOLUME	CHANGE IN KIDNEY VOLUME	REMARKS
			Dog 18.5 kgm.		
gm. per kgm. 0.005	$-\dfrac{204}{96}$	mm. Hg. $-\dfrac{100}{14}$	Increase	cm. $-\dfrac{10.1}{8.5}$ Fall	Curarized and atropinized; fatal. No recovery after epinephrin, cardiac massage and injection of saline (0.9 per cent NaCl)
			Dog 12.5 kgm.		
0.005	$-\dfrac{168}{144}$	$-\dfrac{60}{30}$	Increase	$-\dfrac{7}{6.3}$	
0.005	$\dfrac{144}{144}$	$-\dfrac{70}{50}$	Increase	$-\dfrac{8}{7.5}$	
			Dog 11.0 kgm.		
0.005	Slowed	$-\dfrac{80}{54}$	Increase	$-\dfrac{9.25}{9.1}$	
0.010	$-\dfrac{172}{102}$	$-\dfrac{50}{84}$	Increase	$-\dfrac{10.1}{9.8}$	
0.010	$-\dfrac{(?)}{0}$	$-\dfrac{30}{0}$	Increase (greatly dilated)	$-\dfrac{10.2}{9.8}$	No recovery after injection of tincture of digitalis and epinephrin
			Dog 12.0 kgm.		
0.005	—	$-\dfrac{90}{40}$	Increase	—	Curarized and atropinized
0.005	—	$-\dfrac{40}{24}$	Increase	—	
			Dog 9.0 kgm.		
0.005	—	$-\dfrac{100}{60}$	Increase		
0.0025	—	$-\dfrac{104}{80}$	Increase		
0.005	—	$-\dfrac{110}{70}$	Increase		
0.0025	—	$-\dfrac{94}{84}$	Increase		Vagi cut and atropinized

TABLE 3—*Continued*

DOSE OF CHELIDONIN INTRAVENOUSLY	CHANGE IN PULSE RATE PER MINUTE	CHANGE IN BLOOD PRESSURE	CHANGE IN CARDIAC VOLUME	CHANGE IN KIDNEY VOLUME	REMARKS
			Dog 9.0 kgm. (Cont'd)		
0.005	—	$-\dfrac{94}{64}$	Increase		Vagi cut and atropinized
0.00055	—	$-\dfrac{106}{100}$	Slight increase		
0.005 (papaverin)	—	$-\dfrac{104}{70}$	Slight increase		
0.005 (papaverin)	—	$-\dfrac{64}{54}$	Slight increase		
0.005	—	$-\dfrac{64}{50}$	Increase		
0.011	—	$-\dfrac{50}{30}$	Increase		
0.005	—	$-\dfrac{30}{0}$	Increase		Respiration continued after heart stopped.
			Dog 9.5 kgm.		
0.005	—	$-\dfrac{60}{36}$	Increase	$-\dfrac{7.2}{7.0}$	Curarized
0.005	—	$-\dfrac{36}{30}$	Increase	$-\dfrac{7.1}{7.0}$	Atropinized
0.0075	—	$-\dfrac{34}{24}$	Increase	$-\dfrac{7.1}{7.0}$	
0.005	—	$-\dfrac{34}{29}$	Increase	$-\dfrac{7.2}{7.1}$	
0.010	—	$-\dfrac{24}{20}$	Increase	—	Recovery by administration of tincture of digitalis 0.5 cc. per kgm.
0.005	—	$-\dfrac{50}{34}$	Increase	$-\dfrac{7.2}{7.2}$	
0.010	—	$-\dfrac{30}{24}$	Increase	—	Heart stopped suddenly

* The minus sign (−) means decrease. In the fractions used in the table, the numerator figure represents the observation before and the denominator that after injection of the drug.

blood pressure being at the same level (100 mm.). These differences are attributed to differences in the functional state of the cardiac muscle. As a rule, the effects of small doses were tem-

porary, the pulse rate, blood pressure, cardiac volume and kidney volume returning to their previous levels within two to three minutes. Larger doses (0.01 gram per kilogram) produced lasting effects and even death. The minimal effective dose was about 0.00055 gram per kilo. The effects after section of the
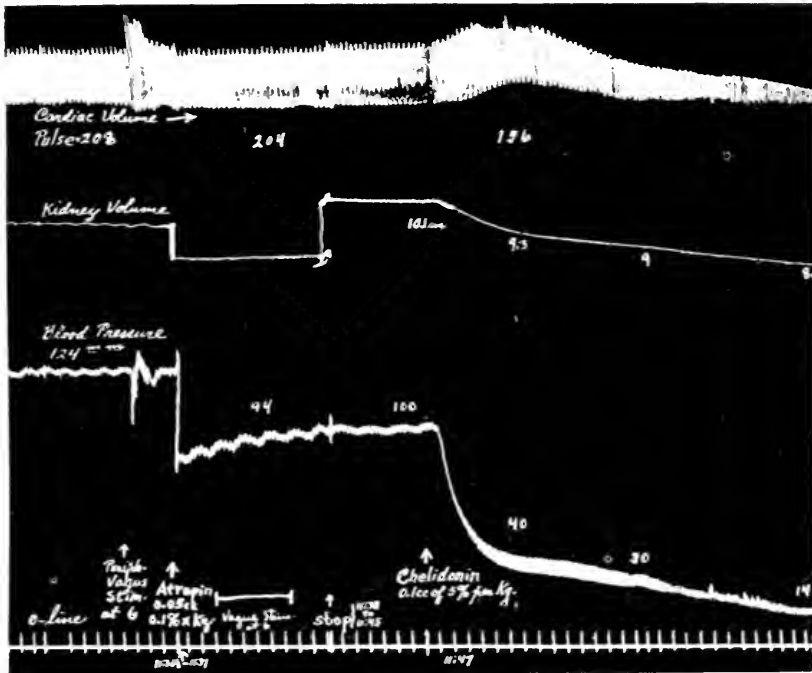
FIG. 1. EFFECTS OF CHELIDONIN ON CARDIAC VOLUME, KIDNEY VOLUME AND BLOOD-PRESSURE OF CURARIZED AND ATROPINIZED DOG (18.5 KGM.).

Cardiac dilatation is recognizable by increase in excursions and rise of cardioplethysmogram. Kidney oncometer readjusted at break in curve. Time: each stroke = five seconds.

vagi and injection of atropin were the same as those on the untreated animals. This indicates that the vagus center and endings are not concerned in the slowing of the heart and fall of blood pressure.

Attempts were made in five different animals to restore the functional activity of the hearts after stoppage by chelidonin, or

at the end of the experiment. The following resuscitative
measures were tried; Tincture of digitalis, epinephrin, injection
of 0.9 per cent sodium chloride, direct electrical stimulation of
the heart, and direct cardiac massage. Practically no success
with these measures was obtained. As a rule, the paralysis was
complete. This indicates that caution is necessary in the use of
chelidonin and related agents (papaverin, narcotin, benzyl
esters, etc). Comparatively speaking, the fatal dose of cheli-
donin is high, and its toxicity, therefore, is relatively low, but
the occurrence of cardiac stoppage as a result of its use must be
regarded as serious, since such hearts can not be easily revived.
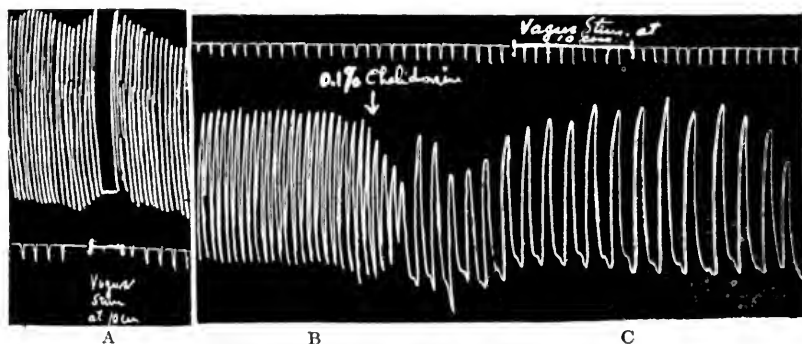
FIG. 2. EFFECTS OF CHELIDONIN ON PERFUSED TURTLE'S HEART

A, Vagus stimulation effective. B, Same heart after perfusion of 0.01 per cent
atropine. C, Vagi inactive; nevertheless chelidonin lowers the tone of and slows
the heart due to direct depression of cardiac muscle. Time: each stroke = five
seconds. Time mark along upper border of B and C.

Mechanism of cardiac slowing. The results on dogs indicate
that the slowing of the pulse with the consequent fall of blood
pressure and kidney volume is of cardiac origin. At the same
time the heart is dilated. H. H. Meyer attributed the slowing
to stimulation of vagus endings, but this can not be the correct
explanation, since the slowing occurs after section of the vagi
and paralysis of the endings by atropin. That the slowing is
due to direct depression of cardiac muscle was shown by per-
fusion of the excised and atropinized hearts of frogs and turtles.

Figure 2 illustrates that chelidonin slows the pulse and lowers
the tone and amplitude of a turtle heart whose vagi were para-

lyzed by atropin. The same effects were obtained on frog's hearts. High concentrations of chelidonin (2 to 5 per cent) caused complete cardiac arrest in the diastolic position. Washing with Ringer's solution removed the moderate effects of small doses, but not always the more pronounced effects of, or stoppage from, large doses. However, the restoration was frequently assisted by such antagonists as morphin, and also epinephrin. The antagonistic effects of morphin and chelidonin and a number of other alkaloids have been discussed in a previous publication (18) and need not be repeated here. All the facts obtained justify the conclusion that chelidonin slows the heart by direct action on cardiac muscle independently of parasympathetic nerve (vagus) connections.

Mechanism of the fall of blood pressure. The chief factor in this is undoubtedly the direct cardiac depression produced by the chelidonin. However, this is not the only factor, for chelidonin also relaxes the peripheral blood vessels. This was shown in a previous publication by the perfusion of frog's extremities, and in this paper by the relaxation of excised blood vessels (carotid artery of pig and aorta of rabbit). The effects on excised vessels will be described in the section on smooth muscle.

According to these results, therefore, the peripheral organ volume should be increased. However, this is not the case. A fall in kidney volume occurred with both small and large doses of the drug. This can only be explained by more effective depression of the heart than arterial muscle by the chelidonin.

This brings up the question of the efficiency of such drugs as chelidonin and papaverin in the treatment of angina pectoris. Apparently the relief would not be attained by direct vascular relaxation, but rather by cardiac depression and the accompanying fall of blood pressure. It may be doubted if this would be the choice method of securing relief in angina pectoris. The usefulness of these drugs might be greater in hypertonus, the high blood pressure of arteriosclerosis, and tachycardias. It should be remembered that cardiac slowing is also obtained after hypodermic injection of chelidonin. This was indicated in the section dealing with symptoms. Therapeutic effects on the circulation, therefore, are obtainable by both intravenous and hypodermic administrations of chelidonin.

4. Effects on respiratory rate and volume

The respiratory effects of chelidonin as indicated by changes in rate after hypodermic injection in different animals appear variable. This was indicated in the section dealing with symptoms. However, complete respiratory effects involve also changes in minute volume, which could not be ascertained by direct observation. Therefore, the effects on respiration were investigated further on rabbits by the Dreser spirometer method.

For this purpose, the animal is fastened to a convenient board without anesthesia. No surgical procedures are used. A truncated rubber cone made by cutting in half an ordinary small sized atomizer bulb is placed over the animal's nose and face. Into the truncated or open portion of the bulb is fitted a T-tube with one end joined by rubber tubing to another T-tube inserted through the stopper of a wide mouthed bottle and containing another long tube dipping in water and arranged in such a way as to constitute a valve. When the attendant's finger rests on the free and open end of the T-tube attached to the cone the animal inspires through the long tube dipping under the surface of the water and expires through the T-tube above the surface of the water. This T-tube is joined by a short rubber tube to a small glass tube bent upwards at the tip so as to fit just within the opening of a large, wide mouthed, inverted burette of 500 cc. capacity, and dipping just under the surface of water in a large pan. All connections with the water valve and burette should be reduced to the shortest possible lengths so as to reduce the dead space to a minimum. The observations are conducted at intervals, allowing the animal to recuperate between observations so as to avoid asphyxial effects which would complicate the results.

The animal being perfectly quiet and breathing without effort, and the water column at the uppermost or zero level of the burette, the observer places a finger at a given signal on the free and open end of the T-tube attached to the cone and counts the respirations for fifteen seconds. Meantime the rabbit is simultaneously displacing the water in the burette. At the end

of fifteen seconds the finger is relaxed and the displacement of water ceases. The volume is read off and expressed in terms of cubic centimeters per minute, which, when divided by the respiratory rate per minute, gives the average volume for a single respiration. The average of at least three constant readings were taken before and after injection of the drug allowing sufficient time for development of its action. In this way observations were made with therapeutic or small, and toxic or large, doses of chelidonin on three rabbits. Three rabbits treated similarly with morphin served as controls. The effects of morphin on minute volume are so well known that controls of this kind would help to eliminate errors of technique or accident in the chelidonin experiments. The results that were obtained with chelidonin are summarized in table 4, and those with morphin in table 5.

Therapeutic dosage. The results obtained indicate that chelidonin and morphin affect respiration in the same direction and that the effects of morphin are more constant than those of chelidonin. Therapeutic doses of morphin reduced the rate by about 25 to 30 per cent and markedly increased the ventilation. The increase in average volume per respiration in the 3 animals studied amounted to from 40 to 225 per cent. On the other hand, much larger doses of chelidonin, or about 12 to 33 times the therapeutic dose of morphin, only moderately reduced (by 20 per cent) the rate and increased (by 38 to 60 per cent) the minute volume of respiration in 2 animals. A dose of chelidonin equivalent to the therapeutic dose of morphin (0.0005 gram per kilo) slowed the rate and minute volume by only about 20 per cent in one animal. An increase of dosage of chelidonin equal to 8 times the therapeutic dose of morphin in the same animal caused a further decrease of less than 10 per cent in both rate and ventilation. According to these results small doses of chelidonin cause very little impairment in the efficiency of respiration. The moderate decrease in respiratory rate is fully compensated by a moderate increase in ventilation. Bearing in mind that a considerable variation exists in the respiratory function of rabbits these effects exerted by chelidonin must be regarded as relatively mild (cf. also fig. 6).

TABLE 4

*Effects of chelidonin on minute volume of respiration**

TIME	RATE PER MINUTE (MEDIAN)	TOTAL MINUTE VOLUME (MEDIAN)	AVERAGE VOLUME PER RESPIRATION
Rabbit 2.0 kgm			
Normal			
		cc.	cc.
	42	408	9.7
Chelidonin sulphate (0.0006 gram per kilogram) double the therapeutic dose of morphine			
End of 30 min.	34	346	10.0 = + 3.0 per cent
1 hr.	32	352	11.0 = +14.4 per cent
2 hrs.	32	428	13.4 = +38.1 per cent
3 hrs	32	408	12.7 = +31.0 per cent
Rabbit 1.7 kgm.†			
Normal			
	52	308	6.0
Chelidonin sulphate, toxic (0.2 gram per kilogram hypodermic)			
End of 30 min.	46	326	7.0 = +11.6 per cent
1 hr.	56	380	6.7 = +30.0 per cent
2 hrs.	42	368	8.7 = +45.0 per cent
3 hrs.	44	420	9.5 = +60.0 per cent
4 hrs.	44	424	9.6 = +60.0 per cent
Rabbit 1.6 kgm.			
Normal			
	36	490	13.6
Chelidonin sulphate (0.0005 gram per kilogram) hypodermically			
17 min.	30	330	11.0 = −20.0 per cent
Chelidonin sulphate (0.05 gram per kilogram) (hypodermically; high therapeutic dose)			
1 hr.	28	320	11.3 = −17.0 per cent
Chelidonin (0.2 gram per kilogram) hypodermically; toxic dose			
2 hrs., 10 min.	32	400	12.5 = −6.0 per cent
Caffein (0.01 gram per kilogram) hypodermically			
3 hrs.	36	600	14.0 = +3.0 per cent
4 hrs.	36	600	14.0 = +3.0 per cent
5 hrs.	32	620	19.4 = +42.0 per cent

* The plus sign (+) means increase; the minus sign (−), decrease.
† Diarrhea at end of 1 hour and 2 hours.

82

TABLE 5

*Effects of morphin on minute volume of respiration**

TIME	RESPIRATION RATE PER MINUTE	TOTAL MINUTE VOLUME	AVERAGE VOLUME PER RESPIRATION
		Rabbit 2.1 kgm.	
		Normal	
	cc.	*cc.*	*cc.*
	42	500	11.5
Morphin (therapeutic dose hypodermically, 0.0005 gram per kilogram)			
End of 1 hr.	29	480	16.1 = +40 per cent
	27	480	16.5 = +43 per cent
Morphin (toxic dose, 0.04 gram per kilogram)			
End of 1½ hrs.	12	188	15.6 = +35 per cent
	16	115	7.2 = −37 per cent
End of 2 hrs.	8	110	13.8 = +20 per cent
Camphorated oil 2 cc. of 20 per cent subcutaneously			
End of 3 hrs.	21	480	22.0 = +100 per cent
		Rabbit 2.0 kgm.	
		Normal	
	59	250	4.3
Morphin (therapeutic dose 0.0005 gram per kilogram)			
End of 10 min.	49	430	8.7 = +102 per cent
Morphin (toxic dose. 0.04 gram per kilogram)			
End of 23 min.	24	0+	0+ = 0
Camphorated oil (0.2 gram per kilogram intraperitoneally)			
End of 58 min.	24	60	2.2 = −50 per cent
	33	113	3.4 = −21 per cent
		Rabbit 1.7 kgm.	
	50	773	15.4
Morphin (therapeutic dose 0.0005 gram per kilogram)			
47 min.	35	620	18.0 = +17 per cent
Morphin (toxic dose, 0.04 gram per kilogram)			
1 hr., 20 min.	12	600	50.0 = +225 per cent
2 hrs., 32 min.	28	535	19.1 = +24 per cent
Camphorated oil (1.5 cc. of 20 per cent) intraperitoneally			
2 hrs., 53 min.	44	700	15.2 = no change; recovery

* The plus sign (+) means increase, the minus sign (−), decrease.

Toxic dosage: The effects of morphin in toxic dosage (0.04 gram per kilo) invariably and markedly reduced the rate and minute volume of respiration. In one rabbit the respiration was so feeble that the volume could not be measured with the apparatus used. On the other hand, a much larger dosage of chelidonin in 2 animals, i.e. 0.2 gram per kilo equivalent to two-thirds the fatal dose, caused no further slowing in the respiratory rate and reduced the minute volume in one and increased it in another animal. However, the tendency in the animal (rabbit 1.6 kgm.) which showed a reduction in minute volume of respiration was actually an improvement as compared with the effects of a preceding smaller dose of chelidonin. Therefore, this effect is actually equivalent to stimulation. Treatment of the morphinized animals with camphorated oil (intraperitoneally) improved the respiratory rate and minute volume in all animals, though variably. Treatment of one of the chelidoninized animals with caffein subcutaneously also improved the rate and minute volume of respiration. These results are not comparable, but they indicate at least that the respiratory centers in these animals were responsive to stimuli despite the nearly fatal doses of the alkaloids that were used.

The only definite conclusion permissible from these experiments on respiration with chelidonin is that the drug is a relatively mild depressant on the rate without serious impairment of the ventilation, which may even be improved. Bearing in mind the variability of this function in rabbits, it seems justifiable to conclude further that the actions of chelidonin in therapeutic doses on respiration are unimportant, possessing in this respect an important advantage over morphin for therapeutic purposes as an analgesic. This confirms in general the absence of marked narcotic effects on the different animals described in this report.

4. Effects on smooth muscle

In a previous paper it was shown that chelidonin depresses the smooth muscle of excised intestine, uterus, stomach and esophagus. The effects of chelidonin are sufficiently powerful to oppose those of such marked augmentors as pituitary extract

and histamin on the uterus, and of barium on intestine and uterus. It was thought desirable to extend the observations to the smooth muscle of other regions, which remained uninvestigated, namely, excised ureter, bladder and blood vessels, and intact bronchi and intestine.

Strips of the excised organs were immersed in Tyrode's solution and the peristaltic contractions recorded on a slow moving kymograph in the usual way. Chelidonin sulphate in different concentrations was added directly into the cylinder containing the organ, using the usual antagonists for analysis of the action. A number of different organs of each kind were studied, but here only representative tracings will be submitted. The methods for studying the effects on intact bronchi and intestine will be described in the section dealing with these organs.

Ureter. Figure 3 illustrates the depressant action of chelidonin on the tonus, amplitude and rate of contractions of dog's ureter. Precisely the same effects were observed on rabbit's and pig's ureter. The effects were not prevented by atropin or abolished by epinephrin. Therefore, the effects are due to depression of smooth muscle directly. The augmentor action of barium was removed by chelidonin.

Bladder. Figure 4 illustrates the depressant action of chelidonin on dog's bladder. The augmentor effects of barium, physostigmin and epinephrin were removed by chelidonin. Since the depression occurs after atropin the effects are due to direct depression of the bladder musculature.

Blood vessels. In a previous paper it was shown that chelidonin relaxes untreated and previously constricted vessels of frogs perfused by the Läwen-Trendelenburg method. In the present report, the observations were extended to excised aorta of rabbit and renal artery of pig. Figure 5 illustrates the chelidonin relaxation of rabbit's aorta previously treated with physostigmin. The tonus augmentor effects of epinephrin and barium were also opposed by chelidonin.

Intact intestine. It was previously (2) shown that chelidonin promptly and markedly depresses the peristalsis of excised intestine, but it does not follow from this that corresponding

FIG. 3. LONGITUDINAL STRIP OF DOG'S URETER IN 150 CC. OF TYRODE SOLUTION

Contractions initiated by barium, abolished by chelidonin; partial recovery by pituitary extract, not by barium, indicating paralysis by chelidonin. Time: each stroke = five seconds.
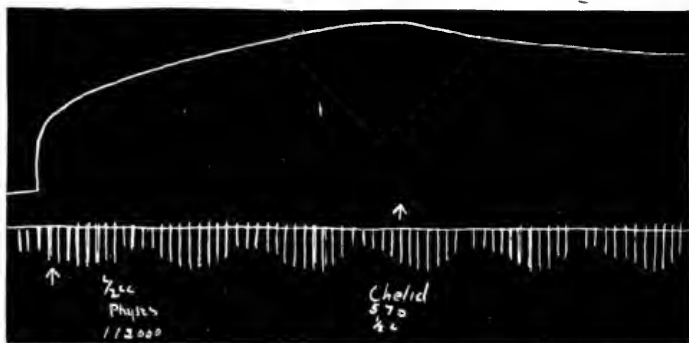
FIG. 4. DOG'S BLADDER; QUIESCENT STRIP IN 50 CC. TYRODE'S SOLUTION

Chelidonin relieves the increase in tonus produced by Physostigmin. Later applications of barium, physostigmin and epinephrin were ineffective. Time: each stroke = five seconds.
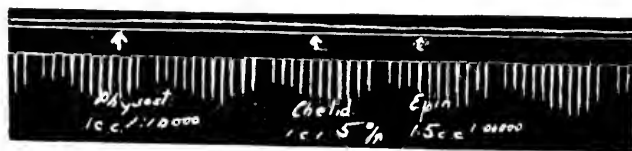
FIG. 5. RABBIT'S AORTA; RING PREPARATION IN 150 CC. TYRODE'S SOLUTION

Chelidonin relieves mild increase in tone by physostigmin; some restoration of tone by epinephrin. Time: each stroke = five seconds.

effects could be obtained on the intact intestine. Inspection of the shaved abdominal wall of untreated and unanesthetized rabbits indicated a slowing of the peristalsis after intravenous injection of chelidonin. This was further tested on decerebrated dogs, using the finger cot method for recording contractions from the intestine. A short (10 cm.) finger cot tied to an L-shaped glass tube and filled with normal saline was introduced into the small intestine, and securely tied at the place of incision. The protruding glass tube was then joined to a tambour by means of rubber tubing using air conduction for recording the contractions on a slow moving kymograph. After introduction of the finger cot into the intestine the abdominal wall was closed by hemostats. Gradually the intestinal contractions appeared, and, when they reached a constant optimum, the experiment was begun. Blood pressure from the carotid was recorded in the usual manner by means of a damped mercury manometer. All injections were made into the saphenous vein. Four animals were used, the injections of chelidonin being repeated several times in each. A number of other agents was used to augment the peristalsis or antagonize the effects of chelidonin. Figure 6 illustrates the inhibition of intestinal peristalsis of untreated intestine.

The results obtained were characterized by a temporary abolition of peristalsis with a slight fall in tone, almost immediately following the injection and during the fall of blood pressure. Peristalsis was resumed just as the blood pressure reached the previous level. These effects lasted about 1 minute and were produced by a dosage of 0.005 gram per kilo, equivalent to about 0.3 gram or 5 grains for an adult man. The effects did not appear to be more pronounced after higher and fatal doses. That the inhibition was not due to the fall of pressure per se was shown by producing a corresponding fall of pressure by hemorrhage without demonstrable changes in the peristalsis. Augmentation of the peristalsis brought about by the injection of barium was also promptly allayed by the injection of chelidonin. As a rule, the marked increase in peristalsis brought about by asphyxia at death was not prevented by the repeated
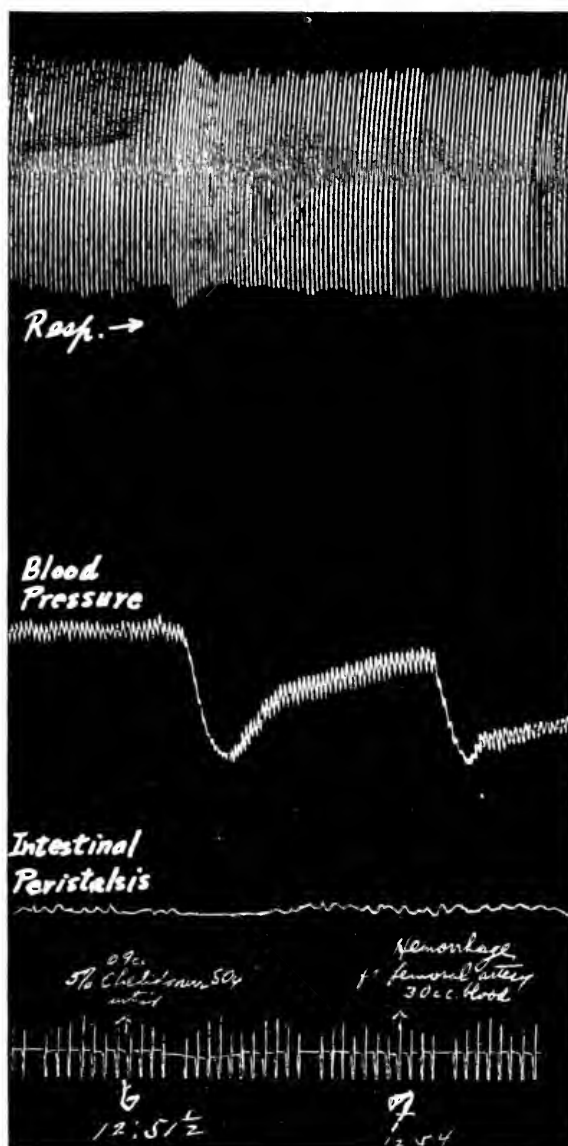
FIG. 6. DOG 15.2 KGM. EFFECT OF CHELIDONIN AND HEMORRHAGE ON INTESTINAL PERISTALSIS, BLOOD-PRESSURE AND RESPIRATION

Time: each stroke = five seconds.

injections of chelidonin previously administered and responsible for the fatal termination. However, in one animal it was shown that the augmented peristalsis produced by moderate and controlled asphyxia can be prevented by chelidonin. The trachea was clamped for five minutes and a control record of the augmented peristalsis was made at the end of this time. The animal was then allowed to recover from the asphyxia while the intestinal peristalsis returned to the previous condition. The asphyxia was then repeated for five minutes in the same way, injecting 0.005 gram chelidonin per kilo intravenously just as the trachea was clamped. The intestine remained quiet and the record showed no contractions, indicating a complete inhibition of the peristalsis. However, in about three minutes after the asphyxia was stopped peristalsis was resumed again. These experiments, therefore, confirm the results obtained on excised intestine and the effects observed by inspection of the abdominal wall of rabbits. However, the effects on intact intestine are not as striking as those on excised intestine. This, of course, will depend to some extent on the functional state of intestinal musculature. Therefore, beneficial therapeutic effects in intestinal colic may be expected.

No attempt was made to compare the effects of chelidonin with other and related agents such as papaverin, the benzyl esters and epinephrin. The consumption of time and animals scarcely warranted the extension of experiments in this direction, and also on the intact uterus, which probably would give the same results as the intestine. Pal (22) has shown that papaverin allays the peristalsis of intact intestine. Macht (19) claims the same for the benzyl esters, but Mason and Pieck (17) obtained only very mild, if any effects whatever, from very large doses of the benzyl benzoate injected intravenously in dogs. On the other hand, extremely small but effective pressor doses of epinephrin produced prompt and profound effects on the peristalsis, greatly lowering the tone and stopping the contractions completely though temporarily. Corresponding negative effects on the pylorus and uterus were also observed by Mason and Pieck with the benzyl benzoate.

Whether chelidonin would be more or less successful than the benzyl esters in allaying intestinal and uterine colic can not be said positively at this time. The successful experiments on dogs here reported with chelidonin as compared with the negative experiments of Mason and Pieck with benzyl benzoate would seem to indicate a degree of superiority in favor of chelidonin. However, comparative experiments and extensive clinical trials would be needed to establish this. Certain advantages possessed by chelidonin (also papaverin) are evident. It is non-irritant, does not cause eructations, can be administered in smaller dosage and in aqueous solution, or dry form in capsules, and it is demonstrably effective in intact animals.

Bronchial effects. Previous observations (2) with chelidonin on bronchi indicated that relaxation of these organs occurs if they are first constricted or their tone is increased. However, there was considerable doubt if the untreated bronchi were also depressed. Therefore, it was thought desirable to extend the observation on the untreated bronchi by a different method, and also on bronchi previously contricted by pilocarpin and histamin.

This was done on decerebrate animals, using the Meltzer pleural cannula, connected with a tambour, which recorded the changes in bronchial tone on a slow moving kymograph. With this method broncho-constriction is recognized by a diminution in the amplitude of excursions maintained by artificial respiration. The effects on the tone of untreated bronchi were studied by observing their response to electrical stimulation of the peripheral ends of the cut vagi. A sufficiently strong current was used to produce a definite though not maximal constriction before the drug was injected. The same stimulus was then applied at intervals after injection of the chelidonin until the bronchial tone returned. When the tone was restored the animal was used for repetition of the same experiment, or observations on previously constricted bronchi by the augmentors. The drugs were injected intravenously into the saphenous vein. The blood-pressure was also recorded from the carotid artery by means of a mercury manometer in the usual way. By this method a very comprehensive demonstration of bronchial changes

TABLE 6

*Bronchial effects of chelidonin**

TREATMENT AND VAGUS STIMULATION	BRONCHIAL CHANGE
Cat 1.5 kgm.	
Peripheral vagus stimulation at 6 cm.†	Constriction and fall of blood pressure
Peripheral vagus stimulation at 5	Constriction and fall of blood pressure
Chelidonin 0.01 gram	None
Vagus stimulation at 5	None for $7\frac{1}{2}$ minutes
Vagus stimulation at 5	Constriction end of 8 min. after injection
Pilocarpin 0.001 gram	Marked constriction
Chelidonin 0.005 gm. in 1 min.	Relaxation slight
Chelidonin 0.010 gm. in 2 min.	Relaxation greater
Epinephrin 0.5 cc. of 1:10,000	Relaxation
Dog 6.0 kgm.	
Vagus stimulation at 10	Constriction
Chelidonin 0.1 gram	None
Vagus stimulation at 10	None
Histamin 0.001 gram	Constriction and moderate rise of blood pressure
Chelidonin 0.01 gram in $1\frac{2}{3}$ min.	Relaxation
Epinephrin 1 cc. 1:1000	Further relaxation and rise of blood pressure
Histamin 0.002 gram	Constriction
Chelidonin 0.01 gram	Relaxation
Dog 5.1 kgm.	
Vagus stimulation at 5	Constriction
Chelidonin 0.014 gram	No constriction; some relaxation
Dog 5.7 kgm.	
Vagus stimulation at 7	Marked constriction
Chelidonin 0.005 gram	Relaxation
Dog 6.0 kgm.	
Vagus stimulation at 7	Marked constriction
Chelidonin 0.015 gram	Slight relaxation
Vagus stimulation at 7, 5 min. later	No constriction
Vagus stimulation at 7, 7 min. later	Definite constriction
Chelidonin 0.01 gram	None
Vagus stimulation at 7, 2 min. later	Definite constriction
Chelidonin 0.01 gram	None
Vagus stimulation at 7, 3 min. later	Ineffective

TABLE 6—*Continued*

TREATMENT AND VAGUS STIMULATION	BRONCHIAL CHANGE
Dog 6.5 kgm.	

TREATMENT AND VAGUS STIMULATION	BRONCHIAL CHANGE
Vagus stimulation at 7	Constriction and fall of blood pressure
Chelidonin 0.012 gram	None
Vagus stimulation at 7 in 40 sec.	No constriction
Vagus stimulation at 7 in 8 min.	Constriction, less than control
Pilocarpin 0.001 gram	Constriction marked
Epinephrin 0.5 cc. of 1:1000	Relaxation in 2 min.
Pilocarpin 0.0005 gram	Constriction marked
Chelidonin 0.012 gram	No effect in 1 min.
Chelidonin 0.012 gram	Relaxation in $\frac{1}{2}$ min.; maximal in 3 min.

* All drugs were injected intravenously.

† The figures for vagus stimulation refer to the position in centimeters on the Harvard inductorium.

can be made on a single dog by appropriate arrangement of the electrical stimulations and agents illustrating effects on the tone of untreated and previously constricted bronchi. Dogs are more suitable than cats and rabbits. The results of the experiments made on 5 dogs and 1 cat are presented in the form of brief protocols in table 6. Figure 7 illustrates some of the effects on dog 6.5 kgm; figure 8, those on cat 1.5 kgm.

The results obtained show definitely that chelidonin depresses the tone of untreated and previously constricted bronchi. Peripheral vagus stimulation of the untreated bronchi (figure 7) was ineffective in all animals after injection of the drug for moderately long though variable periods of time (8 to 10 minutes). Chelidonin also relaxed the bronchi when previously constricted by pilocarpin (illustrated by fig. 8) and histamin. However, epinephrin in much smaller (1/500 to 1/200) dosage relaxed the previously constricted bronchi just as effectively as chelidonin. The dosage of chelidonin used in these experiments ranged from 0.005 to 0.014 gram per kilo intravenously, equivalent to about 0.3 to 0.84 gram for an adult man of 60 kilos. How effective this dosage would be by subcutaneous injection can not be said. In the experiments on animals the blood pressure fell temporarily after each injection of chelidonin, due to direct
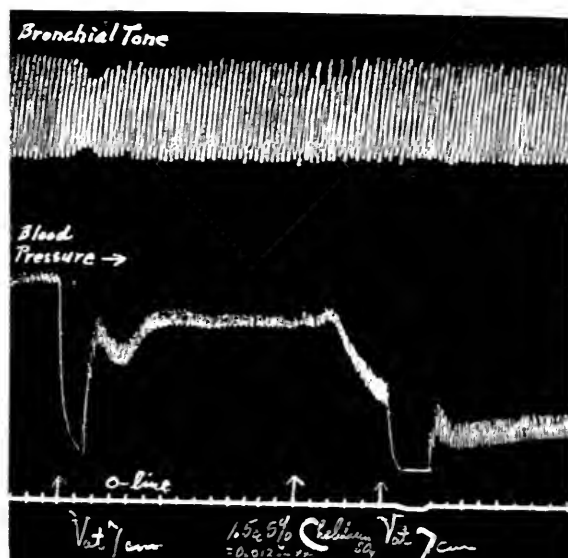
FIG. 7. EFFECT OF CHELIDONIN ON UNTREATED BRONCHI OF DECEREBRATED DOG
(6.5 KGM.)

"V" means electrical stimulation of peripheral ends of both vagi. The
bronchi remained irresponsive to the same stimulus for $6\frac{1}{4}$ minutes after admin-
istration of chelidonin. Time: each stroke = five seconds.
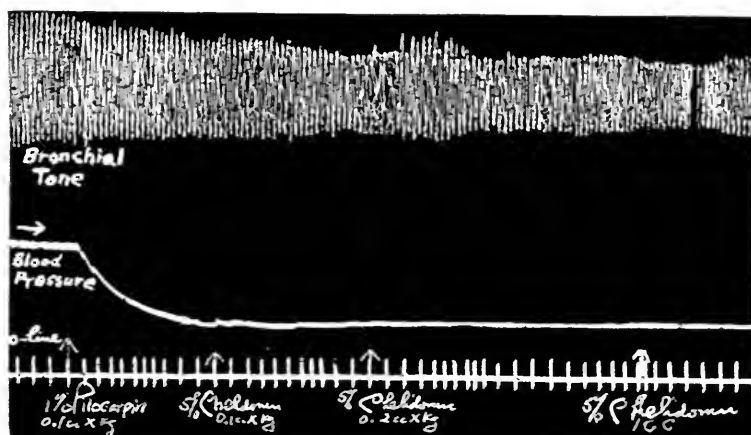
FIG. 8. EFFECTS OF CHELIDONIN ON BRONCHI OF DECEREBRATE CAT (1.5 KGM.)

Previous constriction by pilocarpin relaxed by chelidonin. Time: each
stroke = five seconds.

cardiac depression. If this should occur in human individuals it may be doubted if chelidonin would be the drug of choice in preference to epinephrin in the treatment of bronchial asthma. However, this remains to be determined. Meanwhile, it may be concluded that chelidonin depresses the tone of untreated bronchi and can relieve the spasm produced by such powerful bronchoconstrictors as pilocarpin and histamin.

5. Effects on skeletal muscle and motor nerve

The experiments of H. H. Meyer on electrical stimulation of skeletal muscles from frogs injected with chelidonin indicated depression of these organs. The injection of chelidonin into muscle was found to cause degeneration of the muscle substance. My experiments were made on frog's gastrocnemii and sciatic nerves immersed in 0.1 per cent and 1 per cent solutions of the American variety of chelidonin in Ringer's fluid, using the Claude Bernard arrangement. That is, when the effects on muscle were studied, the muscle was placed in chelidonin and nerve in Ringer's solution, and vice versa when the nerve was observed. The effects were judged by determination of the minimal effective stimuli applied to muscle and nerve directly and at intervals. The results obtained are presented in table 7.

These show that chelidonin depresses skeletal muscle directly, but not the nerve trunk. Stimulation of chelidoninized muscle through the nerve gives a response though feebler than before application of the drug, due to weakening of the muscle by the chelidonin. Depression of the curarized muscle immersed in chelidonin was shown also by direct stimulation through electrodes imbedded in the muscle substance, recording the contractions on a slow moving kymograph. These results agree with the conclusions of Meyer, and with those on cardiac and smooth muscle described in previous sections of this paper. The different kinds of muscle cells are depressed with low and paralyzed by high concentrations of chelidonin, independently of nerve connections. The disintegration effect on skeletal muscle substance agrees with the destructive or dissolution action produced by papaverin on parasites (ameba, paramecia, colpidia and

trypanosomes) observed by others. Nerve substance, however, appears to be much less susceptible to the injurious effects of these agents.

TABLE 7

Effects of chelidonin on skeletal muscle and nerve trunk (frog)

TIME	IN CHELIDONIN 0.1 PER CENT	IN RINGER'S SOLUTION (CONTROL)
Experiment 1		
		Muscle 12 cm. 80°
		Nerve 12 cm. 80°
End of 37 min.	Nerve 12 cm. 80°	Muscle 12 cm. 80°
End of 55 min.	Nerve 12 cm. 80°	Muscle 12 cm. 80°
End of 72 min.	Nerve 12 cm. 80°	Muscle 12 cm. 85°
End of 80 min.	Nerve 12 cm. 80°	Muscle 12 cm. 85°
Experiment 2		
		Nerve 10 cm.
		Muscle 10 cm.
End of 5 min.	Nerve 10 cm.	Muscle 10 cm.
End of 15 min.	Nerve 10 cm.	
End of 40 min.	Nerve 10 cm.	
End of 3 hrs.	Nerve 10 cm.	
Experiment 3		
		Muscle 10 cm.
		Nerve 10 cm.
End of 2 min.	Nerve 10 cm.	
End of 5 min.	Nerve 10 cm.	
End of 12 min.	Nerve 10 cm.	
End of 45 min.	Nerve 10 cm.	
End of 1 hr., 43 min.	Nerve 5 cm.	
End of 3 hrs., 13 min.	Nerve 10 cm.	
Experiment 4		
		Muscle 12 cm. 80°
		Nerve 12 cm. 80°
End of 36 min.	Muscle 12 cm.	Nerve 12 cm. 65°
End of 54 min.	Muscle —	Nerve 12 cm. 70°
End of 70 min.	Muscle 7 cm.	Nerve 12 cm. 70°
End of 80 min.	Muscle 5 cm.	Nerve 12 cm. 5°

TABLE 7—*Continued*

TIME	IN CHELIDONIN 0.1 PER CENT	IN RINGER'S SOLUTION (CONTROL)
	Experiment 5	
		Muscle 10 cm.
End of 7 min.	Muscle 10 cm.	
End of 17 min.	Muscle 10 cm.	
End of 30 min.	Muscle 8 cm.	
End of 43 min.	Muscle 8 cm.	
End of 2 hrs., 12 in.	Muscle 8 cm.	
	Experiment 6 In chelidonin 1 per cent	
		Muscle 12 cm. 75°
End of 5 min.	Muscle 12 cm.	
End of 45 min.	Muscle 0 cm. (dead)	
End of 61 min.	Muscle 0 cm. (dead	

Treatment of strychnin convulsions. The depressant effects of chelidonin on skeletal musculature might be useful for allaying the convulsions of strychnin poisoning. This was tried out on 2 frogs previously injected with convulsant doses of strychnin (0.23 cc. of 0.1 per cent per gram of frog). Forty-one minutes after the convulsions developed, one frog was injected with 0.005 cc. of 5 per cent chelidonin sulphate per gm., equivalent to 0.25 gram per kilo body weight, without demonstrable change in reflex excitability for twenty-four hours, although the convulsions appeared to be somewhat less severe about two hours after the injection. At the end of twenty-six hours, however, they seemed to be as strong as in the beginning. Apparently what little chelidonin effect there was wore off before the action of the strychnin disappeared. The dose of chelidonin used in this frog represented five-sixths of the fatal dose per kilo.

In the other frog, chelidonin equivalent to 1 gram per kilo of body weight or about three times the fatal dose was injected immediately after the first convulsion appeared, using the same dose of strychnin as in the first frog. Six minutes after the injection of chelidonin, convulsions could no longer be elicited. The heart stopped at the end of one hour and twenty-seven

minutes after injection of the chelidonin. In other words, beneficial anticonvulsant effects from chelidonin in strychnin poisoning are obtainable only with a dosage (fatal) which is precluded for therapeutic purposes.

Effects on musculature of mouse's tail. The Straub test (for morphin) was performed on 2 white mice by subcutaneous injection of chelidonin sulphate in the dosage of 0.05 gram and 0.1 gram, respectively, per kilo at the root of the tail. A control mouse was injected with a smaller dose of morphin in the same way. The morphin mouse showed promptly the usual effect of a raised tail, while the chelidoninized mice showed no symptoms whatever. Their tails were not held up at any time during twelve hours, indicating a distinct difference between chelidonin and morphin. Papaverin behaves like chelidonin. According to Macht (20) the tail phenomenon described by Straub is really concerned with effects on the sphincteric musculature of the rectum and bladder, morphin causing a stimulation of this with consequent raising of the tails, while the benzylisoquinolins apparently depress and prevent the tail raising. However this may be the tail raising effect is not strictly characteristic of morphin, since it occurs also after nicotin (Jensen and Rumry (21)), but it is useful to distinguish between morphin and chelidonin.

7. Local anesthetic action

One per cent and 5 per cent solutions of chelidonin sulphate produce a peculiar numbing sensation of the tongue which gradually feels insensitive to moving objects (pin) and to biting. The sensation is like that of a mild degree of anesthesia. This was confirmed on the cornea of two rabbits and one cat.

Five per cent chelidonin was applied by irrigation to the left eyes of the three animals. The right eyes served as controls. A long horse's hair attached to a small handle was touched to the cornea for elicitation of the winking reflex. Five minutes later the winking reflexes were sluggish, but still obtainable, while the right eyes showed active reflexes. At the end of half an hour the left corneas could be touched with considerable pressure and by sharp tapping without elicitation of the winking

reflex. At the end of one hour the winking reflex in all the animals was still sluggish, but obtainable on sharp tapping.

Chelidonin, therefore, possesses a mild local anesthetic action on the tongue, and this is somewhat more pronounced on the cornea. This agrees in general with the local anesthetic properties of papaverin (22) (10 per cent solution).

8. Excretion in urine

Two subjects (L. W. H. and P. D. C.) were given 0.2 gram and 0.1 gram of chelidonin sulphate by mouth in capsules, respectively, and their urines were collected at the end of fifteen minutes, one hour, four hours and twelve hours. The following tests were applied direct to the urines and also to ethereal extracts of the different specimens rendered alkaline. The residues left after evaporation of ether were treated with dilute sulphuric acid and filtered. The following alkaloidal reagents were used; Lugol's solution, Mayer's reagent, picric acid, 1 per cent tannin and phosphotungstic acid. Warren's test (23) for papaverin which gives a positive reaction, i.e., blue color, with chelidonin was also applied. For this the urine or ethereal extractive was acidified with hydrochloric acid, a few drops of potassium ferrocyanide were added, and then a little sulphuric acid with formaldehyde.

The results obtained were as follows: The urines of subject P. D. C., who received 0.1 gram of chelidonin, were entirely negative. Positive tests were obtained with the one-hour specimen only from subject L. W. H., who received 0.2 gram. Apparently, therefore, chelidonin is fairly promptly excreted, but to what extent was not ascertained. The urine of subject P. D. C., gave no tests because probably not enough of the drug was administered. Zahn (24) could not detect papaverin in the organs and excreta of rabbits, cats and dogs after subcutaneous injection.

III. DISCUSSION

The effects of chelidonin on the different functions and organs have been sufficiently discussed in the text. It is only necessary to emphasize certain features that have not been touched upon

thus far, and which will help to appraise properly the usefulness of the drug in pharmacology and therapeutics.

Pharmacologically, chelidonin is almost identical in its actions with papaverin and the benzyl esters. It is distinctly superior to the benzyl esters as far as actual usage goes, because it is practically impossible to obtain results with the benzyl esters uncomplicated by the effects of alcohol or similar solvents necessary for holding them in solution. The benzyl esters are insoluble in water. On the other hand, chelidonin sulphate is soluble and can be prepared in convenient form by dissolving in 2 per cent sodium sulphate. Chelidonin, just like papaverin, is admirably suited for the analysis of smooth muscle phenomena of excised organs and intact animals. It relieves barium contractures of the different kinds of muscles. By the use of these two reagents, namely, barium and chelidonin, it is possible to determine direct muscular depression or stimulation as the case may be. Both drugs act on the different kinds of muscles (smooth, skeletal and cardiac) independently of nerve connections. This has been used successfully in this laboratory for the last three years in demonstrations and teaching exercises for students. Chelidonin, therefore, is a valuable addition to methods of pharmacological analysis. Papaverin, of course, is just as valuable for this, but this drug is virtually unobtainable at present. Furthermore, being obtained from opium whose importation is greatly restricted, it would appear that a substitute for papaverin is desirable. There is no doubt that chelidonin can take the place of papaverin for scientific purposes at least. In this connection it is interesting to note that chelidonin occurs in chelidonium and stylophorum in the absence of the other alkaloids found in opium, yet it resembles papaverin (an opium constituent) very closely in chemical composition and structure. Being obtained from weeds (tetterwort or chelidonium and stylophorum), which grow country wide, and can also be easily cultivated, it is obvious that chelidonin possesses distinct economic advantages over papaverin. It is distinctly desirable that a source of the product in this country be available for use in experimental laboratories and clinics.

The restricted supply of the drug has prevented thus far an extended trial in clinical practice. Hence, it is impossible as yet to speak with certainty as to the therapeutic uses or results. However, chelidonin merits a trial in several different directions, now that its pharmacological actions have been definitely ascertained. It should be useful for the relief of spasm of smooth muscle in various regions, namely, in intestinal and ureteral colic, the uterine colic of dysmennorrhea, bronchial spasm (asthma), hypertonus, high blood pressures of arteriosclerosis, tachycardia, pylorospasm, possibly gastric crises, spastic constipation of lead poisoning, spasm of vesical sphincters, hyperexcitability of uterus during pregnancy to prevent abortion, etc. These various conditions have been favorably influenced by the administration of papaverin, and there is no reason to believe that chelidonin could not do likewise. The single adult dosage should be about 3 to 5 grains administered hypodermically or intramuscularly in the form of a 2 per cent solution of chelidonin sulphate in 2 per cent sodium sulphate (dry salt). Four per cent solutions injected hypodermically give a marked temporary local reaction, though not abscess formation, while 2 per cent solutions cause slight tenderness only. Intramuscularly, 2 per cent solutions give practically no reaction whatsoever.* The drug can also be given by mouth (in capsules) and per rectum. 0.9 per cent sodium chloride can not be used for dissolving chelidonin sulphate since this precipitates the chelidonin as hydrochloride. The drug is now being used in the clinics of Cleveland hospitals and it is hoped that the results may be available for publication in due time.

IV. CONCLUSIONS

1. A comprehensive résumé of the older literature with an extended study of the pharmacological actions and uses of chelidonin is presented.

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* I am indebted to Dr. R. W. Scott of the Cleveland City Hospital and Dr. C. Burns Craig of the Neurological Institute, New York City, for recent observations along this line.

2. The fatal dose of chelidonin for several species of animals is about 0.3 gram per kilo subcutaneously, or equivalent to about 1.8 gram for a 60 kilo man. The adult therapeutic dose should be about 0.2 gram or 3 grains hypodermically or intramuscularly, using 2 per cent chelidonin sulphate dissolved in 2 per cent sodium sulphate.

3. Systemically, chelidonin produces moderate depression with some tendency to narcosis, slowing of the pulse and variable and unimportant changes in the respiration.

4. Slowing of the pulse is accompanied by a diastolic tendency of the heart, fall of blood pressure and kidney volume due to direct depression of cardiac muscle, independently of parasympathetic nerve connections.

5. In rabbits, small and large doses of chelidonin cause only a moderate and variable slowing of the respiration with corresponding increase in ventilation, resembling morphin to some extent qualitatively, but quantitatively the effects of chelidonin are much weaker.

6. Chelidonin depresses smooth muscle of various excised organs independently of nerve connections, namely, all portions of the alimentary tract, ureter, bladder, uterus and artery. The same depression occurs in intact animals (bronchi, intestine, and presumably also uterus).

7. Chelidonin antagonizes the augmentor effects of barium, histamin, and pilocarpin on intestine, uterus and bronchi; of pituitary extract on intestine and uterus; and of epinephrin on pregnant uterus.

8. Skeletal muscle is also depressed directly by chelidonin, but not the motor nerve trunk.

9. Chelidonin in high concentrations exerts a mild degree of local anesthesia on the tongue and stronger on the cornea.

10. Chelidonin has been detected in urine one hour after administration.

11. Pharmacologically, chelidonin is a useful reagent for the analysis of muscular phenomena, especially of smooth muscle.

12. Therapeutically, chelidonin merits a trial for the relief of spasm of smooth muscle anywhere, namely, in intestinal and

ureteral colic, uterine colic of dysmenorrhea, bronchial spasm, hypertonus, hyperexcitability of uterus in pregnancy, tachycardia, pylorospasm, etc.

13. Economically, chelidonin possesses distinct advantages over papaverin, which is virtually unobtainable at present. It has the further advantage over the benzyl esters that it can be administered subcutaneously and intramuscularly and is not disagreeable by mouth.

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THE PHYSIOLOGICAL ACTION OF N-METHYLHIS-TAMINE AND OF TETRAHYDROPYRIDO-3.4-IMINA-ZOLE ("IMIDAZOLISOPIPERIDIN" OF FRÄNKEL)

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The suggestion of a connection between histamine and the pituitary active principles, which has appeared from time to time in the literature dealing with the investigation of the latter, has been recently revived by the publications of Abel and his colleagues. The existence of certain resemblances between the two—physiologically in their actions on the plain muscle of certain organs, chemically in the apparently close association of the pituitary activity with a substance giving Pauly's diazoreaction—has been familiar to one of us since he participated in the work which brought the activity of histamine to notice. Numerous unpublished attempts, in past years, to follow this hint have proved uniformly fruitless, and the failure of our recent attempt to follow up Abel's highly suggestive indications is described in a preceding paper. Nevertheless it seemed useful to take any opportunity of examining and describing the action of derivatives of histamine.

I. N-METHYL-HISTAMINE (β-METHYLAMINO-ETHYLGLYOXALINE)

This substance was available as the dihydrobromide, kindly submitted to us for examination by Prof. F. C. Pyman and Mr. R. G. Fargher, who had prepared it by decarboxylation of the corresponding N-methyl-histidine. The salt was dissolved so as to give a solution containing 1 mgm. of the free base per cubic centimeter and compared, on the blood pressure of a cat under ether and on the isolated uterus of the virgin guinea-pig, with a solution of histamine, similarly reckoned as free base.

Blood pressure

N-methyl-histamine has a depressor action on the arterial pressure of the cat, qualitatively similar to that of histamine, but much weaker. Figure 1 shows that 0.01 mgm. of histamine has an action definitely greater than that of 1 mgm., and not perceptibly different from that of 2 mgm. of N-methyl-histamine. The ratio of activities is, therefore, 1 : 200.

Uterus

As shown by the curves in figure 2, N-methyl-histamine has much less than one-fiftieth and somewhat more than one-hundredth of the activity of histamine on the guinea-pig's uterus. The comparison was not made within narrower limits, but it can be safely concluded, from inspection of the record, that the ratio of activities is in the neighborhood of 1 : 80 or 90. It can be stated quite definitely that N-methyl-histamine, while it is very much weaker than histamine in both directions, has a higher ratio of uterine stimulant to vascular depressor activity than histamine itself.

II. TETRAHYDROPYRIDO-3.4-IMINAZOLE

("IMIDAZOLISOPIPERIDIN" OF FRÄNKEL)

In a recent paper Fränkel and Zeimer (1920) have described the preparation of a substance of the formula
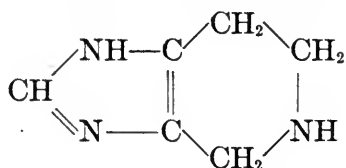
which they call "Imidazolisopiperidin." They state that it has a similar physiological action to that of histamine but is much more powerful. "Es hat sich entsprechend der theoretischen Voraussetzung mit bezug auf die Wirksamkeit herausgestellt, dass die Substanz das so wirksame proteinogene Amin, das β-imidazolyl aethylamin, weit übertrifft und gleichsinnig wirkt."
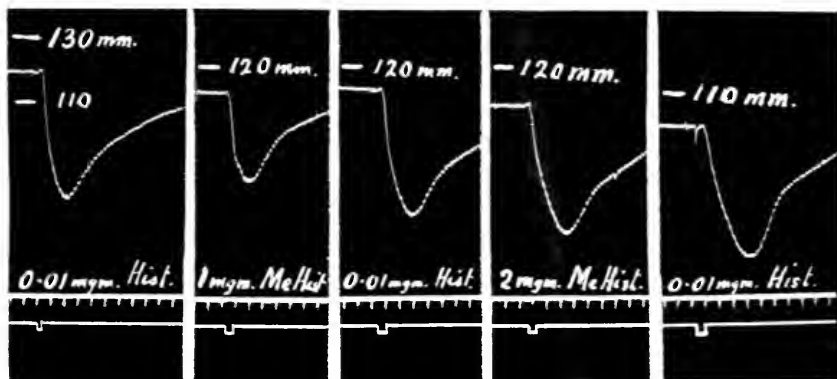
FIG. 1. BLOOD PRESSURE OF CAT UNDER ETHER
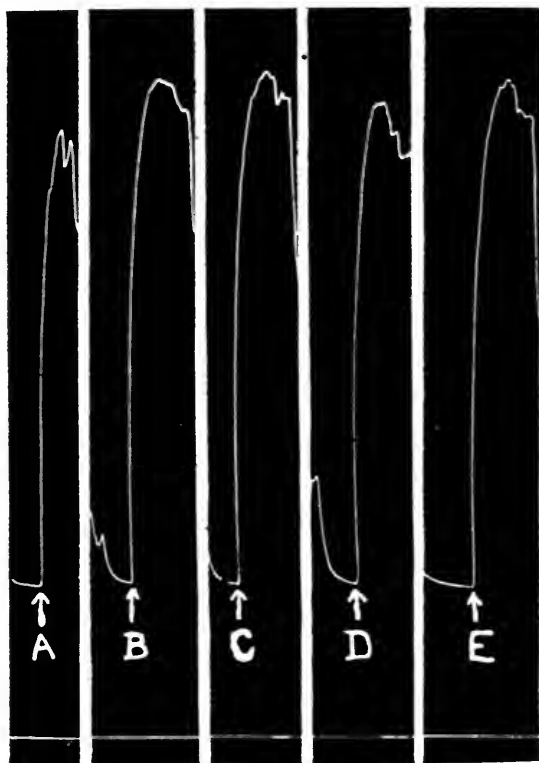
FIG. 2. HORN OF GUINEA-PIG'S UTERUS SUSPENDED IN 80 CC. RINGER SOLUTION

At *A* added 0.25 mgm. methylhistamine; at *B* added 0.005 mgm. histamine; at *C* added 0.5 mgm. methylhistamine; at *D* added 0.005 mgm. histamine; at *E* added 0.5 mgm. methylhistamine.

In view of the well known intense activity of histamine this statement was of great interest and consequently the preparation of a small amount of "imidazolisopiperidin" was undertaken.

Fränkel and Zeimer prepare "imidazolisopiperidin" by the action of methylal on histamine hydrochloride dissolved in concentrated hydrochloric acid at 100°C. They evaporate the reaction-mixture with water to remove excess of hydrochloric acid and then recrystallise the residue from alcohol, obtaining the dihydrochloride of "imidazolisopiperidin," with a reported melting point of 253°C.

In the experiment described in this communication histamine diphosphate was dissolved in concentrated hydrochloric acid and the solution was treated at 100°C. with an excess of methylal much greater than that in Fränkel's synthesis. From the reaction mixture the dipicrate was prepared. After recrystallisation from water this was converted into the dihydrochloride which was twice recrystallised from 96 percent alcohol. The dihydrochloride thus obtained had a melting point of 276° to 278°C. (uncorrected.)

The melting point of Fränkel and Zeimer's dihydrochloride is at least 25°C. below this value, which suggests that their compound was not pure.

The experience gained in making this substance leads to the belief that, prepared according to Fränkel and Zeimer's directions, it would be heavily contaminated with unchanged histamine dihydrochloride, since, although over twice as much methylal as Fränkel took was used in the condensation, unchanged histamine was isolated from the reaction mixture and identified. That small amounts of histamine dipicrate crystallised out along with the recrystallised "imidazolisopiperidin" dipicrate was obvious from the fact that the residue from the recrystallisation of the dihydrochloride had a typical histamine-like action on the isolated uterus of the guinea-pig, much greater than that of the twice recrystallised specimen.

The name "imidazolisopiperidin" for the substance described is not very appropriate. It is not strictly a piperidine derivative. A more correct chemical name would be tetrahydropyrido-3-4-iminazole.

Preparation of tetrahydropyrido-3-4-iminazole dipicrate

Two and one-half grams histamine disphosphate, dissolved in in 15 cc. concentrated hydrochloric acid, were heated under a reflux condenser on the water bath and during a period of two hours 6.2 cc. methylal were slowly added through a fine capillary projecting through the condenser almost to the surface of the solution. After all the methylal had been added heating was continued for two more hours. A further 2.5 grams histamine diphosphate were treated in the same way. The solutions from these two condensations were combined and, after the addition of water, evaporated in vacuo to a syrup. The syrup was taken in up water, and neutralised to congo red with sodium hydroxide. To it was added a solution of 7.6 grams picric acid in hot water, to which had been added 34 cc. N. NaOH. An immediate crystalline precipitate was formed, and when the solution was cold the crystals were filtered off and dried at 90°C. Yield: 8.15 grams; melting point 208°–210°C. (uncorrected.)

This dipicrate was recrystallised from boiling water, from which it separated as glistening bright orange plates. The yield was 7.45 grams and it melted at 210° to 214°C. (uncorrected).

Preparation of tetrahydropyrido-3-4-iminazole dihydrochloride

7.45 grams of the dipicrate were dissolved in hot water and 45 cc. concentrated HCl were added. On cooling picric acid crystallised out and was removed by filtration. The residual picric acid in the solution was extracted with ether and the aqueous solution was then evaporated in vacuo to dryness. The crystalline residue was taken up in water and again evaporated to dryness to remove excess of HCl. The dihydrochloride was then twice recrystallised from 96 per cent alcohol and 1.43 gram of the salt, crystallising in long prismatic needles, was obtained. It had a melting point of 276°–278° C. (uncorrected) and was analysed giving the following results:

Water of crystallization. 0.1284 gram air-dried substance heated at 110°C. lost 0.0114 gram.

per cent

Found.. 8.88
Calculated for 1 mol. H_2O.. 8.41

Nitrogen (Kjeldahl). 0.1170 gram anhydrous substance gave 17.8 cc. $N/10$ NH_4OH.

per cent

Found.. 21.3
Calculated... 21.4

Chlorine. 0.0803 gram anhydrous substance gave 0.117 g. AgCl.

per cent

Found.. 36.1
Calculated... 36.2

ISOLATION OF HISTAMINE DIPICRATE FROM ORIGINAL REACTION MIXTURE

The mother liquor from the preparation of the dipicrate was allowed to stand for two days in the cold room. A small quantity of compact, orange-colored prisms crystallized out on the sides of the beaker. They were filtered off and dried, 0.14 gram being obtained. This picrate melted at 232° to 236°C. (uncorrected) and the melting point remained unchanged when the substance was mixed with pure histamine dipicrate.

This dipicrate was converted into the dihydrochloride in the usual way and compared with an equivalent solution of histamine diphosphate on the isolated uterus of the guinea-pig. Identical contractions were caused by equal doses of the two solutions.

PHYSIOLOGICAL TESTS OF PURE TETRAHYDROPYRIDO-3-4-IMINA-ZOLE DIHYDROCHLORIDE

The product, when finally purified, was compared with histamine for its activity on the cat's blood-pressure and the guinea-pig's uterus.

Blood-pressure

Figure 3 shows that tetrahydropyrido-3-4-iminazole has practically no action on the blood-pressure. A dose of 0.01 mgm. of histamine causes the usual evanescent fall of 30 mm., while a dose of 30 mgm. of tetrahydropyrido-3-4-iminazole (thpi), i.e., 3000 times the dose, causes a doubtful trace of fall, followed by a
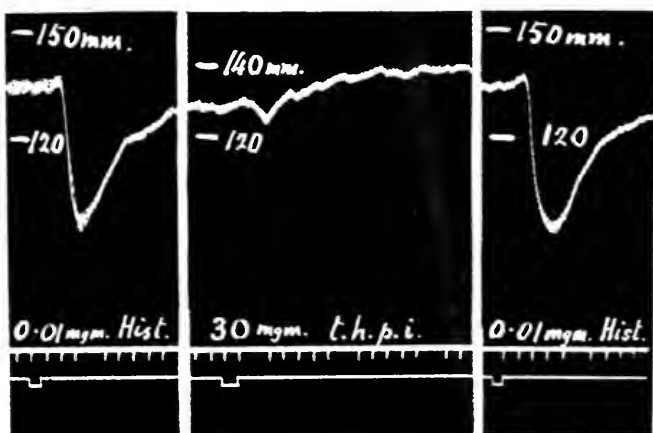
FIG. 3. BLOOD PRESSURE OF CAT UNDER ETHER
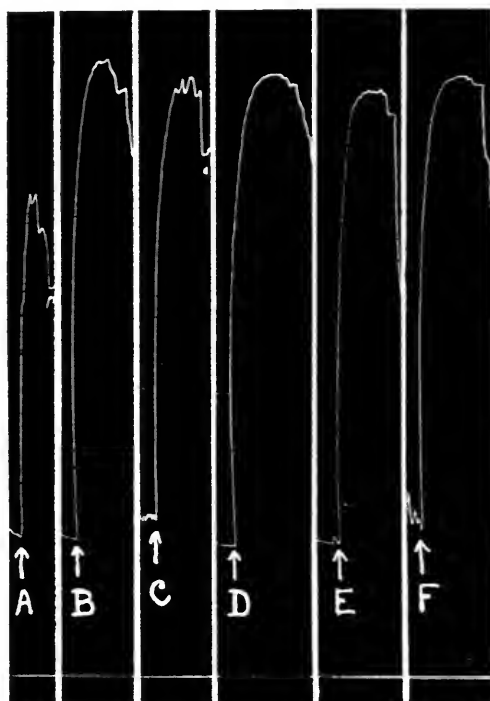
FIG. 4. HORN OF GUINEA-PIG'S UTERUS SUSPENDED IN 80 CC. RINGER SOLUTION

At *A* added 2.5 mgm. tetrahydropyrido-3-4-iminazole; at *B* added 0.005 mgm. histamine; at *C* added 5.0 mgm. tetrahydropyrido-3-4-iminazole; at *D* added 10.0 mgm. tetrahydropyrido-3-4-iminazole; at *E* added 0.005 mgm. histamine; at *F* added 7.5 mgm. tetrahydropyrido-3-4-iminazole.

109

very small and rather persistent rise.   On the uterus the activity is more definite, though again of a much lower order than that of histamine.

Uterus

Figure 4 shows that 5 mgm. of tetrahydropyrido-3-4-iminazole produce less, 10 mgm. somewhat more, 7.5 mgm. about the same as 0.005 mgm. of histamine.   That is to say, the ratio of activities is about 1:1500.   Fränkel's statement that his product had an action of the same type as that of histamine, but far more powerful, can only be explained on the supposition that his preparation was seriously contaminated with histamine, as, indeed, his description of its isolation would suggest, and that the quantative statement was based on general impression rather than careful comparison.

SUMMARY

N-Methylhistamine is found to have one two-hundredth of the activity of histamine on the blood-pressure, and about one eightieth of the activity on the uterus.

Fränkel and Zeimer ascribe a histamine-like physiological action to tetrahydropyrido-3-4-iminazole("imidazolisopiperidin") and state that it is much more powerful than histamine.   Contrary to this statement it has been found that the above compound has only one fifteen-hundredth of the activity of histamine on uterine muscle, and practically no action on the blood-pressure.

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RESPONSE TO DRUGS OF EXCISED BRONCHI FROM NORMAL AND DISEASED ANIMALS

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While countless experiments have been made by numerous investigators on excised and surviving organs of various animals and the different conditions affecting the survival or life of such preparations outside the body have been carefully determined, such studies, remarkably enough, have been confined almost exclusively to normal organs or tissues. Indeed most writers describing such experiments emphasize the importance of employing perfectly normal and fresh organs for the purpose which they have in view. Little attention has been paid, so far as the authors are ·aware of to the behavior in vitro of freshly excised tissues from organs more or less diseased.

The present authors have been engaged for some time in the study of the action of various drugs on isolated bronchi, the results of which studies are described in a subsequent paper. In connection with this work however, certain differences in the behavior between bronchial tissue obtained from normal and pathological lungs have been noted which it was deemed desirable to describe briefly in a separate communication.

METHOD

The action of drugs on the bronchi was studied by the use of bronchial rings, a method first described by Trendelenburg (1) and later employed by other experimenters. While Trendelenburg employed the organs of the ox; in the present investigation all the experiments were made with the bronchi of the pig.

This was done chiefly because such material could be most conveniently obtained in a perfectly fresh condition, some times within a half hour after the killing of the animal, from an abattoir situated within a stone's throw of the laboratory. Immediately after slaughtering the animal the lungs are excised and, from these, pieces of bronchi, on an average 1 cm. in diameter are cut out and washed free from mucus with Locke's solution. The bronchi are then cut into rings 0.5 cm. wide, the rings are cut open and the bronchial cartilage removed, by very careful dissection, from the combined layers of bronchial muscle and mucosa. The bronchial strips thus obtained are suspended in a small glass chamber filled with 25 or 30 cc. of warm oxygenated Locke's solution. One end of the bronchial strip is fixed at the bottom of the chamber while the free end is attached to the short arm of a lever, the long arm of which is arranged to write on a slowly moving kymograph. The small chamber containing the bronchial strip suspended in the Locke's solution is immersed in a water bath and the temperature is kept constant at 38°C. For the study of the reaction of bronchial muscle to physiological or pharmacological agents it is essential in the case of the bronchi, just as in the case of excised arterial rings or strips (2), to overcome first the excessive tonic contraction of the preparation occurring after the death of the animal. To do this a weight varying from 1 to 5 grams is suspended from the longer lever and the preparation is stretched for a period varying from fifteen to thirty minutes. After that the "stretching weight" is taken off and a very light "lifting weight" is suspended in its place. The lifting or balancing weight is so adjusted that the bronchial muscle writes a perfectly horizontal line on the kymograph. This stage having been reached the preparation is ready for study. The effects of drugs are investigated by the introduction of warm solutions directly into the chamber in which the bronchial muscle is suspended and soon after this the bronchial muscle responds by contraction or relaxation as the case may be.

BEHAVIOR OF NORMAL AND PATHOLOGICAL PREPARATIONS

When a bronchial preparation obtained from a fresh and normal animal is suspended according to the method described above it will respond quickly and sharply to treatment with pharmacological agents. Among the drugs which act most powerfully in this way are pilocarpin and muscarin, which produce a broncho-constrictor effect on the one hand, and atropin and papaverin, which produce a broncho-dilator effect on the other hand.

The pig, however, is an animal which is especially subject to a great many pulmonary diseases. Thus it is known that tuberculosis (3) is very prevalent among them and their lungs often show also broncho-pneumonic, catarrhal and other pulmonary lesions. Pneumonia as a primary condition is not of frequent occurrence in swine, but is usually associated with, or secondary to some other disease. Broncho-pneumonia is very common and is most often of bacterial origin, but may also follow parasitic disease, especially in young animals, which are particularly susceptible to the strongylus. Broncho-pneumonia is one of the characteristic lesions found in swine plague, the infection being due to B. suisepticus. It is also frequently found secondary to hog cholera. Other causative factors of this affection in swine are the tubercle bacillus, B. suipestifer and various types of micrococci, as can be gathered from such publications as those of Hutyra and Marek (4), McFadyean (5) and others.

Such pathological conditions can be generally recognized on gross examination as well as after microscopical study. The bronchial preparations, moreover obtained from such lungs were found to behave very differently from normal bronchial preparations. Even before suspension in Locke's solution such bronchial preparations give evidence of abnormal characteristics. The mucosa is seen to be inflamed and is of a deeper red color, while the muscularis of such preparations feels distinctly much less elastic and is more friable than that obtained from normal lungs. On treatment with drugs such bronchial preparations respond abnormally. When the bronchial infection is not of an

intense character, the preparations still respond to the action of drugs, but in a much lesser degree. Bronchial preparations obtained from badly affected lungs however, fail to respond to the action of drugs altogether.

The authors have made systematic observations of the lungs whenever obtained from the slaughter-house and compared the anatomical appearance with the behavior in vitro of bronchial

FIG. 1   FIG. 2

FIG. 1. BRONCHUS OF PIG

Normal; showing marked contraction produced by 1 mgm. of pilocarpin hydrochloride and relaxation by atropin sulphate 1 mgm.
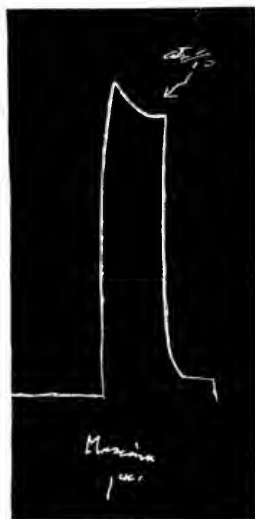
FIG. 2. NORMAL BRONCHUS OF PIG

Marked contraction produced by 1 cc. of saline extract of Amanita muscaria (= about 25 mgm. of dry fungus); and relaxation by atropin sulphate 0.1 mgm.

preparations obtained from them. In this way it was found almost invariably (after the technique had been mastered) that perfectly normal bronchi yield active and sensitive bronchial preparations; whereas slightly diseased bronchi yield preparations which are much less responsive to the action of drugs, and still more pathological bronchi fail to respond to the action of such drugs altogether. The accompanying figures will illustrate the points mentioned above.

Figure 1 shows the response of a perfectly normal bronchus to 1 mgm. of pilocarpin hydrochloride in 30 cc. Locke's solution and the marked relaxation produced in the same preparation by 1 mgm. of atropin sulphate.

FIG. 3. BRONCHUS OF PIG

At M, 1 cc. of muscaria extract (= 25 mgm. of dry fungus) produced a small contraction.   Atropin sulphate 5 mgm. produces relaxation.
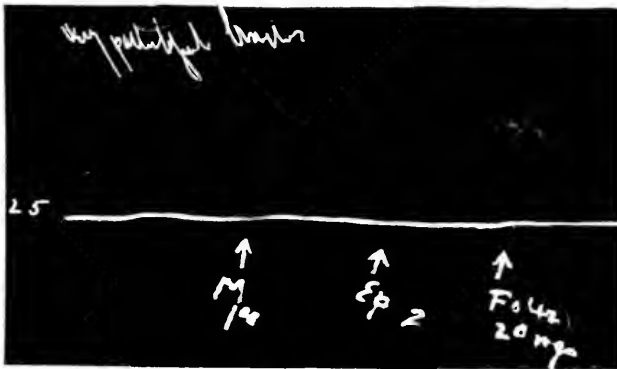
FIG. 4. DISEASED BRONCHUS OF PIG, FRESHLY EXCISED

No response to muscarine (M), epinephrin ($Ep.$) or barium chloride ($BaCl_2$).

Figure 2 shows the immediate and powerful contraction of a normal bronchus elicited by the addition of 1 cc. of a saline solution of fly fungus, Amanita muscaria (equal to 25 mgm. dry fungus).   Complete relaxation is produced in this case by 0.1 mgm. of atropin sulphate.
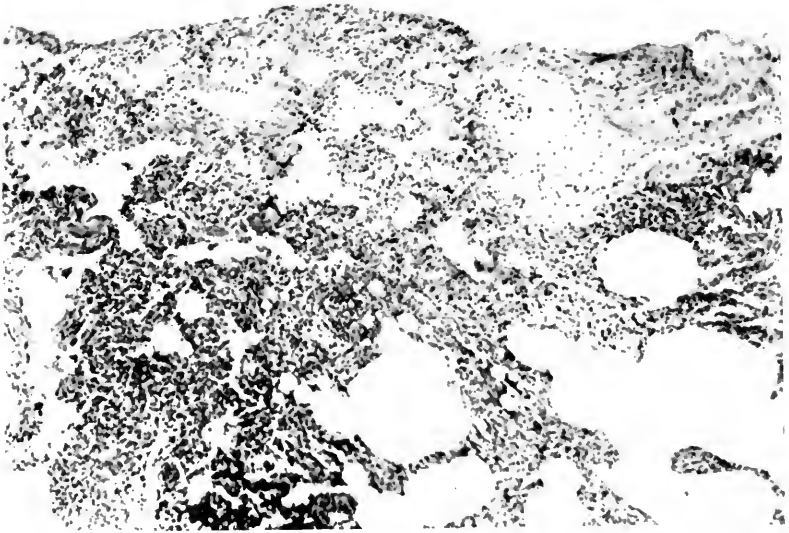
Fig. 5. Section of Pig's Lung Showing Consolidation and Inflammatory
Process. × 75

To correspond with figure 4
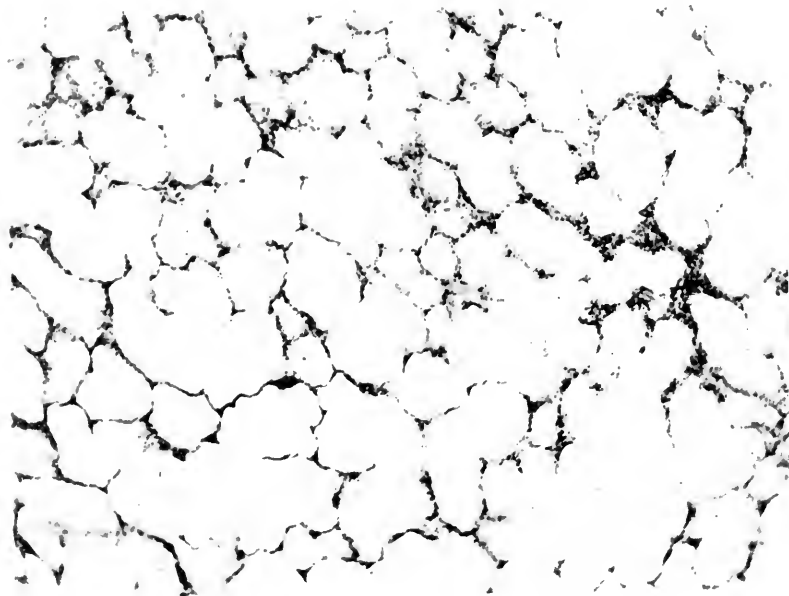
Fig. 6. Section of Normal Lung of Pig. × 75

To correspond with figure 1

Figure 3 shows the response of a bronchial preparation obtained from a slightly pathological lung. The bronchial mucosa in this case was slightly injected and the lung was mildly congested but showed no consolidations. While the preparation was exactly of the same size as that in figure 1 the response to muscarin on the one hand and atropin on the other, was much less marked.

In figure 4 a bronchial preparation was obtained from a lung which showed definite inflammatory lesions with patches of consolidation. A microphotograph obtained from the vicinity of the bronchus shows a pathological condition resembling that of broncho-pneumonia. (The authors not being familiar with veterinary pathology, do not venture to give a more definite diagnosis.) (See figure 5.) It will be seen in figure 4 that whereas the preparation was a perfectly fresh one obtained immediately after slaughtering the animal, the bronchial muscle failed to respond either to muscarin (M) or epinephrin ($Ep.$, 20 mgm.) nor even to barium chloride ($BaCl_2$ 620 mgm.).

Figure 6 shows a microscopic section of a normal pig's lung such as would correspond to the bronchial preparation shown in figure 1.

DISCUSSION

The difference in response between the normal and pathological bronchi just noted cannot be ascribed to the lack of freshness in the preparations or decomposition of the same outside of the body. For in the first place the material was obtained immediately after slaughtering the animals and in the second place normal bronchi will continue to live and respond to drugs when kept in Locke's solution on ice, even twenty-four hours after the death of the animal. The failure to respond on the part of the pathological preparations must therefore be regarded as due to an impairment in the normal physiological properties of the bronchial muscles in question, in other words to a change of a distinctly pathological physiological character. While such a finding is not at all remarkable or unexpected, the authors nevertheless deem it worth emphasizing because the bulk of pharmacological work that is being done concerns itself with

normal animals or tissues almost exclusively, and the fact is often lost sight of as for instance in the case of digitalis, that the effects of drugs on pathological organs may give not only quantitative but even qualitative differences from those obtained from normal organs. One of the authors (M) has noted very similar results while working on isolated ureters and vasa deferentia and the above remarks offer at least a partial explanation of the failure to obtain physiological response from specimens obtained in the autopsy room. Even in the case of tissues (ureters, vas deferens, etc.) obtained from the surgical operating room after nephrectomies, vasectomies, etc., physiological response could be obtained only from bits of perfectly normal tissue. The above difference between normal and pathological bronchial muscle may perhaps also serve as at least a partial explanation of the failure of some patients suffering from asthma to respond to treatment with powerful drugs as illustrated by figure 4. Thus a powerful bronchial spasm will generally give way to an injection of epinephrin and yet if the spasm be accompanied by an inflammatory or catarrhal condition of the bronchi, it is possible that such an inflammatory condition might interfere with the normal response of the bronchial muscle to that drug.

SUMMARY

1. Normal preparations of bronchial muscle respond promptly and quickly to the action of certain pharmacological reagents.

2. Bronchial preparations obtained from pathological lungs even in a very fresh condition, respond to the action of the same drugs much less readily or not at all.

3. This difference in the response between normal and pathological bronchial preparations would seem to indicate that in the latter case there is an impairment of the normal physiological properties of bronchial muscle.

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EPINEPHRINE HYPERGLYCEMIA. II

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In a preceding communication (1) we have presented evidences supporting our belief that epinephrine hyperglycemia is not a direct result of circulatory stasis in the liver subsequent to vasoconstriction but that hyperglycemia and acid production are independent manifestations of epinephrine action. The next logical step in the pursuit of this problem is a study of the conditions under which epinephrine acts as a glycogenolytic agent.

Ritzmann (2), it will be recalled, concluded from his infusion experiments that epinephrine effects glycogenolysis only when vasoconstriction is produced. This led him to conclude that stasis was causative in the mechanism and not as we have recently been led to believe parallel or quite incidental to glycogenolysis. Furthermore Ritzmann concluded that intravenous injections of epinephrine were more effective than subcutaneous injections. Underhill (3) called attention to the apparent inadequacy of Ritzmann's experimental evidence in that Ritzmann compared unanesthetized subcutaneously injected animals to those urethanized and intravenously injected. Consequently while no doubt is cast on the accuracy of Ritzmann's observations, it would not appear fair to compare results obtained from such dissimilar conditions of experimentation. Underhill proceeded to correct the dissimilarity of experimental conditions by avoiding the use of any anesthetic whatsoever and concluded that subcutaneous injections, dose for dose, were more efficient than when given intravenously. In both sets of investigations urinary sugar was used as a criterion of epinephrine hyperglycemia.

121

This again, to our minds, seems unfair since the appearance of sugar in the urine involves the passage of sugar through the kidney which does not accurately reflect the sugar status of the blood, since rather large variations in blood sugar concentration may occur without any significant amount of sugar appearing in the urine (Pollak (4)).

A study of the blood directly is certainly one step closer to the local glycogenolytic process than urinary examinations. This may in turn be too remote or too involved to give ultimate conclusions, yet we feel that such a study is a closer approach and less subject to objection than a study of urinary sugar. Consequently we have approached this question by examination of the blood, in our endeavor to determine some of the conditions under which epinephrine produces glycogenolysis. We are furthermore aware that a study of hyperglycemia may not be an entirely safe criterion of glycogenolysis for it is conceivable that considerable dextrose may be carried from the liver to other organs, there to be deposited or to be oxidized or in some unrecognized fashion rendered undetectible.

The use of phloridzin to produce a condition of renal permeability for sugar is not without objection since phloridzin undoubtedly does some damage other than in the kidney. As a consequence we so far known of no criterion safer than hyperglycemia.

Our study involves three propositions, namely:

1. Is epinephrine as a glycogenolytic agent stable in the circulation or does it have but a transient existence in the blood stream as does epinephrine as a pressor agent.

2. Is there any relationship between absorption of epinephrine as a glycogenolytic agent and the resultant glycogenolysis, in other words, does glycogenolysis occur only so long as epinephrine is being absorbed.

3. Is there any essential physiological difference between epinephrine administered subcutaneously and epinephrine administered intravenously.

In an attempt to answer the first two questions the following experiments were performed. Epinephrine in from 0.5 to 1

mgm. doses were injected into the loose subcutaneous tissues on the dorsum of the hind foot. After an interval of ten to twenty minutes a tight constrictor of rubber bands was placed above the heel joint in such a way as to stop all circulation of blood in the foot. Specimens of blood were taken just prior to application of the constrictor and again ten, thirty, and sixty minutes afterwards when after the last specimen was taken the constrictor was removed. After a time another specimen of blood was taken to observe the effects of the release of the ligature and return of the circulation.

As can be seen from table 1, the development of hyperglycemia is cut short by application of the constrictor. The sugar concentration rapidly declined to normal values within one-half to one hour. Release of the constrictor permitted further absorption of epinephrine with consequent increase in sugar mobilization (see Meltzer and Meltzer (5) for corresponding finding in regard to vasomotor responses).

This series of experiments we believe leads inevitably to the conclusion that glycogenolysis proceeds paru passu with absorption of epinephrine from the region of injection and furthermore the glycogenolytic process does not much if at all outlast this peripheral absorption, and thus indicates the rapid removal of the glycogenolytic epinephrine from the circulating blood.

Next, we have attempted to compare the glycogenolysis from epinephrine when given subcutaneously to that given intravenously. For the latter, we have used small volumes of adrenalin, either the full strength preparation (1:1000) or this diluted five five times (1:5000), injected very slowly throughout a period of from ten to twenty minutes. The condition of the heart beat was closely observed throughout the period of injection. When vagal beats appeared the rate of injection was slowed, but no attempt was made at this point to entirely avoid cardiac slowing. Specimens of blood were taken at the end of the period of injection and again after one-half and one hour.

These values are compared to sugar estimation after the same quantities of epinephrine were injected subcutaneously.

TABLE 1

Effect of sequestration of adrenalin injected area on the course of hyperglycemia in the rabbit

EXPERI-MENT NUMBER	TIME	HEMOR-RHAGE	BLOOD SUGAR	NOTES
	1921	*cc.*	*mg./cc.*	
5a	February 7, 4:15 p.m.			Ligated foot just above heel joint
	4:45 p.m.	2.5	1.04	
	5:30 p.m.	2.5	0.94	
5b	April 2, 2:05 p.m.	2.5	0.98	
	2:10 p.m.			Constrictor applied
	3:06 p.m.	2.5	0.98	
	3:08 p.m.			Constrictor released
	3:55 p.m.	2.5	1.02	
	4:03 p.m.			0.5 cc. adrenalin, subcutaneously
	5:00 p.m.	2.5	2.51	
5c	April 4, 8:45 a.m.	2.5	0.98	
	8:50 a.m.			Constrictor applied
	10:00 a.m.	2.5	1.08	
	10:02 a.m.			Constrictor released
	11:00 a.m.	2.5	1.08	
	11:08 a.m.			0.5 cc. adrenalin, subcutaneously
	12:00 m.	2.5	2.51	
6	February 8, 10:10 a.m.			Constrictor applied
	10:15 a.m.	2.5	1.25	
	10:18 a.m.			Injected 0.5 cc. adrenalin subcutaneously into dorsum of ligated foot
	11:15 a.m.	2.5	1.13	
	11:16 a.m.			Constrictor released
	12:05 p.m.	2.5	2.55	
7	February 8, 2:05 p.m.			Injected 0.5 cc. adrenalin subcutaneously into dorsum of hind foot
	2:15 p.m.			Constrictor applied just above heel
	2:20 p.m.	2.5	2.05	
	2:45 p.m.	2.5	1.81	
	3:15 p.m.	2.5	1.33	

TABLE 1—*Continued*

EXPERIMENT NUMBER	TIME		HEMORRHAGE	BLOOD SUGAR	NOTES
	1921		*cc.*	*mg./cc.*	
		3:15 p.m.			Constrictor released
		4:00 p.m.	2.5	2.31	
		6:00 p.m.	2.5	1.88	
8	February 9,	3:15 p.m.			Injected 0.75 cc. adrenalin into dorsum of hind foot
		3:35 p.m.			Constrictor applied
		3:39 p.m.	2.5	2.13	
		4:09 p.m.	2.5	1.51	
		4:36 p.m.	2.5	0.98	
		4:40 p.m.			Constrictor released
		5:40 p.m.	2.5	1.61	

Table 2 demonstrates the quick rise in sugar during the course
of the intravenous injection, with a decline in values paralleling
those obtained by ligation of the leg after subcutaneous injection.
The degree of hyperglycemia produced in the series of intravenous
injections is of the same order as that in the subcutaneous series
with this difference: the intravenous series developed a rapid
rise in sugar to decline from the end of the injection period, while
in the subcutaneous injection series there is a slow rise but more
prolonged course before the fall if no constrictor was used while
with a constrictor the decline paralleled that from intravenous
injections.

Gramenitzki (6) reported results quite different from ours in
that in his series he observed a progressive increase in blood sugar
from shortly after cessation of injection to an hour or more.
In our series there occurred during this time practically complete
return to normal values. We have seen instances in our own
experience in which such a course as Gramenitzki described
occurred which we ascribe to a too sudden injection of epinephrine with consequent cardiac injury. Furthermore Gramenitzki's
injections involved large volumes, in some instances equal to
or exceeding one-half of the total normal blood volume. This
hydremic plethora may be a considerable factor, though we are

TABLE 2

Intravenous injection of adrenalin

ANIMAL NUMBER	TIME	HEMOR-RHAGE	BLOOD SUGAR	NOTES
	1921	*cc.*	*mg./cc.*	
9	February 15, 2:50 p.m.			Injected 0.2 cc. adrenalin* into ear vein. Time, 10 minutes
	{3:00 p.m.			
	{3:10 p.m.	2.5	1.74	
	3:40 p.m.	2.5	1.41	
	4:10 p.m.	2.5	1.07	
10	February 16, {2:55 p.m.			Injected 0.2 cc. adrenalin into ear vein. Time, 10 minutes
	{3:05 p.m.			
	3:15 p.m.	2.5	1.88	
	3:45 p.m.	2.5	1.41	
	4:15 p.m.	2.5	1.13	
11	February 23, {9:42 a.m.			Injected 0.1 cc. adrenalin into ear vein. Time, 10 minutes
	{9:52 a.m.			
	10:59 a.m.	2.5	1.41	
	10:28 a.m.	2.5	1.26	
	10:55 a.m.	2.5	1.08	
12	February 24, { 9:54 a.m.			Injected 0.2 cc. adrenalin into ear vein. Time, 10 minutes
	{10:04 a.m.			
	10:15 a.m.	2.5	2.04	
	10:42 a.m.	2.5	1.61	
	11:08 a.m.	2.5	1.16	
13	February 25, { 9:50 a.m.			Injected 0.2 cc. adrenalin into ear vein. Time, 23 minutes
	{10:13 a.m.			
	10:20 a.m.	2.5	1.71	
	10:53 a.m.	2.5	1.41	
	11:23 a.m.	2.5	1.26	
14a	February 25, 10:35 a.m.			Injected 0.2 cc. adrenalin subcutaneously
	10:43 a.m.	2.5	1.10	
	11:09 a.m.	2.5	1.51	
	11:37 a.m.	2.5	1.88	

TABLE 2—*Continued*

ANIMAL NUMBER	TIME		HEMOR-RHAGE	BLOOD SUGAR	NOTES
	1921		*cc.*	*mg./cc.*	
14b	March 28,	10:30 a.m.			Injected 15.0 mgm. atropine sulphate, subcutaneously
		11:19 a.m.			
		11:39 a.m.			Injected 0.2 cc. adrenalin into ear vein. Time, 21 minutes. No sign of vagal change in heart during injection
		11:50 a.m.	2.5	1.76	
		12:30 p.m.	2.5	1.26	
		1:45 p.m.	2.5	1.13	

* Adrenalin diluted in approximately 2 cc. Ringer's solution in this series.

inclined to ascribe the discrepancies of Gramenitzki's and our results to injury to the heart or other tissues subsequent to a too rapid injection rate. We cite his experiment xxiv in which there was injected 0.3 mgm. epinephrine in 30 cc. solution during a period of three minutes, while in our experiments the shortest time was ten minutes. It is only fair to add that in a minority of Gramenitzki's experiments he injected through a ten minute period and obtained results (aproximating ours) which he took to be exceptional and consequently based his conclusions on the results obtained by sudden injections. By carefully controlling the injection rate we have been entirely successful in observing prompt recession of the hyperglycemia.

Owing to the fact that circulatory disturbances follow intravenous injections of concentrated epinephrine solutions, the evidence is perhaps not entirely unequivocal that such circulatory disturbances, such as vasoconstriction and cardiac irregularity may not be a considerable factor in the resultant glycogenolysis. Dynamically, vasoconstriction may be in part counterbalanced by a powerful dilator such as nitroglycerine (Cameron (7)). With this type of control in mind we have administered nitroglycerine and epinephrine simultaneously. The antagonism is far from satisfactory since the nitroglycerine effects diminish

TABLE 3

Intravenous injections of mixtures of adrenalin and nitroglycerine

ANIMAL NUMBER	TIME	HEMOR-RHAGE	BLOOD SUGAR	NOTES
	1921	*cc.*	*mg./cc.*	*cc.*
15	March 10, 10:05 a.m.	2.5	1.13	
	⌠10:10 a.m.			Injected intravenously:
	⌡10:30 a.m.			Spirits nitroglycerine . 3.5
				Adrenalin 0.4
				Ringer's solution. 3.1
	10:35 a.m.	2.5	2.15	
	11:13 a.m.	2.5	1.96	
	11:35 a.m.	2.5	1.74	
16	March 11, 8:30 a.m.	2.5	1.22	
	⌠9:08 a.m.			
	⌡9:20 a.m.			Injected intravenously:
				Spirits nitroglycerine . 2.50
				Adrenalin 0.25
				Ringer's solution 2.25
	9:36 a.m.	2.5	2.51	
	10:30 a.m.	2.5	1.88	
17	March 12, 9:15 a.m.	2.5	1.13	
	⌠9:27 a.m.			
	⌡9:45 a.m.			Injected intravenously:
				Spirits nitroglycerine . 4.00
				Adrenalin 0.25
				Ringer's solution 3.75
	10:00 a.m.	2.5	2.26	
	10:35 a.m.	2.5	2.26	
	11:25 a.m.	2.5	1.81	
18	March 12, 3:45 p.m.	2.5	0.94	
	⌠3:50 p.m.			
	⌡4:12 p.m.			Injected intravenously:
				Spirits nitroglycerine . 4.
				Ringer's solution 4.00
	4:22 p.m.	2.5	1.74	
	5:15 p.m.	2.5	1.08	
19	March 14, 10:45 a.m.	2.5	0.90	
	⌠11:00 a.m.			
	⌡11:15 a.m.			Injected intravenously:
				Alcohol 4.0
				Ringer's solution 4.0
	11:30 a.m.	2.5	0.90	
	11:56 a.m.	2.5	0.94	

progressively. Blood pressure control experiments prove that for a single short period injection the antagonism is quantitative, yet in prolonged injections epinephrine effects begin to predominate. Nevertheless vagal manifestations are for the most part abolished when the rate of injection is sufficiently slow.

Here in table 3 it is to be observed that glycogenolysis is as marked if not more so than in those experiments in which epinephrine is given alone.

The use of nitroglycerine requires of itself control study. Nitroglycerine alone, slowly injected caused hyperglycemia though not equal in grade to that produced by the simultaneous use of epinephrine and nitroglycerine. The alcohol of the nitroglycerine preparation (official spirits of nitroglycerine) has when given alone no significant influence. The cause of nitroglycerine hyperglycemia is obscure, but may be referable to prolonged low blood pressure or to some other undetermined action. At least it can be said that nitroglycerine with epinephrine is as effective, if not more so than the same amount of epinephrine given alone. In this case vagal action is at no time serious and from blood pressure experiments it was found that pressure changes are of minor grade and much less than that given by epinephrine alone.

Vagal action is abolished by atropine. Epinephrine administered after atropinization exerts its usual glycogenolytic action, consequently the vagal manifestations of epinephrine intravenously administered cannot be held responsible for the resulting hyperglycemia.

DISCUSSION

A transient existence of epinephrine in the circulating blood stream is proven by the cessation of glycogenolysis at ligation or sequestration of the injected area and also by the cessation after discontinuance of intravenous injections.

Therefore we believe the evidence is complete that epinephrine affects the glycogenolytic mechanism by continued bombardment or stimulation. Furthermore the hypothetical existence of a different form or combination of epinephrine in the blood

subsequent to absorption from subcutaneous injections is not required as a postulate since no discrepancies appear to exist. At any rate there is no evident difference between the epinephrine as a glycogenolytic agent when given subcutaneously than when given intravenously. The assumption of altered epinephrine in the one case is no more valid than for the other. We do not deny the possibility that epinephrine as a pressor agent may differ from epinephrine as a glycogenolytic agent, yet the recovery curves strongly indicate a marked similarity between the instability or absorption from the blood stream of epinephrine studied from the standpoint of pressor changes or of glycogenolysis.

Subcutaneous injection of epinephrine is safer, easier and withal more efficient as a glycogenolytic agent than an intravenous injection, not on account of different substances acting, but owing to a slower and more continuous absorption over a considerable period of time. Pollak, it may be noted, came to essentially this same conclusion in his study of hyperglycemia and glycosuria subsequent to intravenous as well as to subcutaneous injections.

Since epinephrine subcutaneously administered in proper amounts produces glycogenolysis without demonstrable systemic changes in blood pressure, the conclusion is necessary that the threshold (on the basis of minimal requisite concentration) for glycogenolysis is lower than for pressor action. While the threshold is lower for glycogenolysis, the stimulus must be continuously applied since the criterion for such action is remote, i.e., changes in total blood sugar concentration which of necessity is late and a rather coarse measure while pressure changes are acute and easily detected. Theoretically a single adequate stimulus should be followed by *some* glycogenolysis which effect would certainly be beyond our present means of detection in whole blood.

Since subcutaneous injections of epinephrine may be given with no demonstrable change in blood pressure yet producing marked changes in sugar, and since intravenous injections also produce marked changes in sugar, accompanied by pressure

changes, unless controlled by a dynamic antagonist which last does not abolish, in our experience, epinephrine hyperglycemia, it appears to us that we are quite justified in considering that pressure changes are incidental to glycogenolytic action. The presence or absence of blood pressure changes subsequent to intravascular or subcutaneous injections can be readily explained on the basis of differences in rate of absorption, and until it can be positively shown that such is not the case we shall feel warrented in concluding that the different means of administration determine differences in quantity rather than quality of absorbed epinephrine.

CONCLUSIONS

1. Epinephrine as a glycogenolytic agent like epinephrine as a pressor agent has only a transient existence in the circulating blood.

2. Epinephrine glycogenolysis occurs only paru passu with the inflow of epinephrine into the blood, be it from subcutaneous tissues or from direct injection.

3. There is no evidence of the existence of any essential difference between the glycogenolytic action of epinephrine given intravenously and that given subcutaneously.

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QUANTITATIVE PATHOLOGICAL STUDIES WITH ARSENIC COMPOUNDS

I. THE INFLUENCE OF FASTING AND VARIOUS DIETS ON ARSPHENAMINE POISONING AND THE COMPARATIVE TOXICITY OF ARSPHENAMINE, NEO-ARSPHENAMINE AND PARA-OXY-META-AMINO-PHENYL-ARSENOXIDE

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INTRODUCTION

The quantitative studies in chemotherapy published, and in progress by Prof. Carl Voegtlin and his collaborators (1920 and 1921) dealing with the trypanocidal action of antimony and arsenic compounds stimulated the following quantitative pathological studies with arsenic compounds. This is the first paper of a series which will deal with the quantitative action of various drugs on the tissues of the host under varying conditions. It will be seen from this paper that fasting and various diets markedly influence the lesions produced by the intravenous administration of sodium arsphenamine. The action of arsphenamine and neoarsphenamine upon the animal organism differs greatly when considered from a pathological point of view. The kidney lesions produced by arsphenamine, neoarsphenamine and p-oxy-m-amino-phenyl-arsenoxide are described and compared on a quantitative basis. Experiments are included which show the effect of therapeutic doses of mercury on the kidney following toxic doses of disodium arsphenamine. Liver necrosis with jaundice has been produced by the intravenous administration

133

of lethal doses of disodium arsphenamine. Finally a method is proposed for the standardization of the rat for the biological testing of drugs.

EXPERIMENTS WITH RATS

a. The influence of fasting and various diets on the toxicity of arsphenamine

The influence of fasting and diet upon the toxicity of other drugs has suggested to us that these factors may also play an important rôle in arsphenamine and neoarsphenamine poisoning. On account of the considerable variation in the individual susceptibility of animals to arsphenamine poisoning the number of experiments required in order to obtain reliable data on the effects of diet is necessarily very large. For this reason we have used the albino rat and report the results obtained with over six hundred animals. It is clearly shown that a diet of white bread, rolled oats and milk, fed in the days preceding the intravenous administration of arsphenamine, exerts a marked protective action against the toxicity of the drug.

The great difference in the individual susceptibility of patients has already stimulated the following clinical observations: Westrope (1916) stated that a milk diet given twenty-four hours prior to the administration of arsphenamine reduces the toxic effects of the drug to a minimum, and the patients suffer little or no discomfort. Bailey and MacKay (1920) observed that patients with toxic jaundice following the administration of neoarsphenamine and mercury may have an increased amount of blood urea, which they attribute to the high protein content of the diet and to the effect of exercise.

An extensive literature has been developed concerning the influence of fasting and diets on the toxicity of drugs. Lo Monaco and Trambusti (1894) found that well-nourished dogs and rabbits were less resistant to phosphorus than poorly nourished animals. It was also observed that the degenerative changes in the liver and kidneys were much more pronounced when the animals received water during the fasting experiments. Mansfield (1905) carefully reviewed the previous literature on the effects of fasting on the toxicity of drugs, and studied the influence of inanition on narcosis. He found that starving rabbits

were more susceptible to chloral hydrate, paraldehyde and morphine
than were well nourished animals.   On the other hand, the toxic effect
of ethyl alcohol, amylene hydrate and ethyl urethane was not increased
by starvation.   Ellinger (1905) observed that rabbits fed on oats and
given cantharidin subcutaneously showed a severe hemorrhagic nephri-
tis, evidenced by bloody urine and a marked albuminuria.   The reaction
of the urine was invariably acid.   Rabbits fed on turnips and given
the same amount of cantharidin subcutaneously showed only a faint
trace of albumen, if any, and the reaction of the urine was always
alkaline.   Beddard (1908) suggested the therapeutic use of dextrose
in the treatment of chloroform necrosis and also the advisability of
of feeding it before chloroform anaesthesia.   His suggestion is based
upon Rosenfeld's view that the poisoned liver cells can utilize carbo-
drates far better than proteins or fats.   Wells (1908) states that feeding
of dextrose to animals lessens the amount of fatty degeneration which
results from phosphorus poisoning, and presumably it should have
some similar effect in chloroform poisoning.   Foster (1910) studied
the influence of different proportions of protein in the food on resist-
ance to the toxicity of ricin and on recuperation from hemorrhage.
He considered the ricin experiments inconclusive and that recuperation
from hemorrhage depends more upon the breed of the dog and individual
idiosyncrasy than upon the amount of protein in the diet.   Salant
and Rieger (1910) studied the effect of starvation on the toxicity of
caffein.   The fatal dose for rabbits starved four or five days was about
30 per cent less than for well fed animals.   Hunt (1910) made careful
observations on the effects of a restricted diet and of various diets
upon the resistance of animals to certain poisons.   The most striking
results were obtained with acetonitrile.   Guinea-pigs and mice kept
upon a restricted diet were more resistant to acetonitrile than those
kept on an unrestricted diet.   Mice fed upon articles of food such as
enter largely into the daily diet of man varied greatly in their resistance.
He was able to alter the resistance of the animals at will and to over-
come the effects of one diet by combining it with another.   The pro-
tective action is attributed by Hunt, in part, to a specific effect upon
the thyroids.   Dextrose, oatmeal, liver and kidney increased the resist-
ance while eggs, milk, cheese and various fats greatly lowered the
resistance.   Several glands, notably prostate and testes, exerted a
beneficial effect similar to but much less marked than that of thyroid,
while thymus, parathyroids and suprarenals had either no effect, or an
effect opposite to that of thyroid.   Diet also had a profound effect on

propionitrile poisoning. The toxicity of morphine was distinctly but not markedly affected. Salant and Rieger (1912) studied the protective action of diet against the toxicity of caffein. It was found that a high protein diet for the adult dog tended to greater resistance of the animal to caffein, and similarly, the growing dog tolerated larger quantities of caffein on a milk diet than on a diet of meat. Salant and Smith (1914) observed that carrot-fed rabbits resisted much larger doses of sodium tartrate than those which received oats and cabbage. More than twice the fatal dose could be given without producing symptoms. Opie and Alford (1914 and 1915) investigated the influence of diet upon necrosis caused by hepatic and renal poisons. Rats were used as experimental animals. A rich carbohydrate diet, it was found, protects the parenchymatous cells of the liver or kidneys from necrosis caused by chloroform, phosphorus, potassium chromate and uranium nitrate. Chloroform and uranium nitrate were much more toxic to animals which had received a diet consisting in great part of fat than to those which had received meat. The susceptibility to intoxication with phosphorus was not increased by a diet of fat. The toxicity of potassium chromate was not greater after a diet of fat than after a diet of meat. Graham (1915) demonstrated that the feeding of carbohydrates to adult dogs increases their resistance against liver necrosis by chloroform. Salant and Nelson (1915) found the toxicity of oil of chenopodium for cats and rabbits which had received cottonseed or cocoanut oil was diminished at least 50 per cent in some cases. Evidence was also obtained that a diet rich in carbohydrates has a similar effect on rabbits to which the essential oil was administered. Salant and Bengis (1917) observed that the administration of the oil of chenopodium by mouth and subcutaneously may be followed by renal disturbances in rabbits fed exclusively on oats. No evidence of impaired kidney function was obtained when the same, or even larger, doses were administered to rabbits on a diet of carrots. Salant and Wise (1918) demonstrated the protective action of diet against zinc malate poisoning. The rabbits which received an exclusive carrot diet exhibited a decidedly greater resistance to zinc poisoning than those which were fed oats alone. The glycosuria and albuminuria were also frequently absent in the carrot-fed rabbits. Salant and Swanson (1918) published further articles on the influence of diet on the toxicity of sodium tartrate and the protective action of diet against tartrate nephritis. Rabbits, cats and rats were used as experimental animals. The sodium tartrate was administered subcutaneously. The diets arranged in order of their protective value are as follows: For

rabbits, carrot leaves, young carrots, sweet potatoes, table beets, sugar beets, winter carrots, cabbage, hay, oats and glucose. Starved rabbits showed the least resistance. The effect of diet in tartrate poisoning in cats and rats was not constant. Cats showed a decreased resistance when starved. Carbonates increased resistance probably by the neutralization of organic acids in the blood. They concluded that the favorable effects of some diets on the toxicity of tartrates might be due to several factors, among them inhibition of bacterial activity in the intestines, vitamines, or unknown constituents that might be present in some diets. The elimination of phenolsulphonephthalein was decidedly inhibited in rabbits receiving a diet of oats as compared with those on a carrot diet. Davis and Whipple (1919) published a series of articles on the liver injury effected by chloroform anaesthesia as influenced by fasting, various diets, drugs and chemical agents. They clearly show that no single theory so far advanced will explain the peculiar protective action of certain food substances against the liver injury of chloroform anaesthesia. They show further, that it is a reaction of the liver cells, not of substances circulating in the blood stream. Dogs were used as experimental animals. Maximal liver injury was obtained when the animals were starved or placed on a fat diet. Sugar, and diets rich in carbohydrates when fed in the days preceding chloroform anaesthesia exerted a marked protective action against liver injury. Skeletal muscle and heart muscle had a slight protective action while kidney, liver and brain exerted a considerable amount of protection. Desiccated thyroid, given alone, or in combination with foods, did not modify the expected chloroform injury. Glucose, or cream, given intravenously during chloroform anaesthesia did not modify the effect of the drug on a starved animal. Epinephrin, given either subcutaneously or intramuscularly, and quinine sulphate when administered in the days preceding chloroform exerted a marked protective action against liver injury. They point out that these facts should not be lost sight of in the management of human cases in which chloroform is indicated. They recommend liberal amounts of carbohydrates and milk for at least two days preceding the anaesthesia and emphasize the fact that it is dangerous to give chloroform to man or animal whenever a fasting period has preceded the administration of the anaesthetic. Voegtlin, Hooper and Johnson (1920) published experiments which seem to indicate that dogs on a mixed or meat diet are more resistant to trinitrotoluene poisoning than animals fed on bread and milk. The animals fed on bread and milk as a rule showed a more acute and severe anaemia and died sooner.

Experimental

Approximately all the deaths due to arsphenamine poisoning in rats occur within two days after the injection. Many of the animals die within twelve hours and show a characteristic pathological picture. The lungs are usually distended, congested, oedematous and may contain petechiae. The right heart is dilated. The liver, spleen, kidneys and the mucosa of the small intestine are usually engorged. Frequent interstitial hemorrhages are also found in the kidneys. The most characteristic and constant pathological finding in rats that die on the second day is the necrosis of the epithelial lining of the uriniferous tubules. Central hyaline necrosis of the liver lobules may occur but this is extremely rare (only ten animals in over six hundred injected). The detailed pathological findings will be described later and compared with those found in animals poisoned with neoarsphenamine and p-oxy-m-amino-phenyl-arsenoxide.

Tables 1, 2 and 3 briefly summarize the results obtained in the feeding and fasting experiments. Table 1 shows the percentage of rats that died within two days, table 2 shows the percentage of rats that died acutely within twelve hours, and table 3 shows the percentage of rats that lived twelve hours or longer and showed kidney necrosis. In order to place the experiments on a quantitative basis, the doses given in the tables represent the number of cubic centimeters of a 1:100 arsenic equivalent solution per kilo body weight. A 10 cc. 1:100 arsenic equivalent solution contained 24.88 mgm. arsphenamine. From nine to forty-three rats were injected with each dose given in the tables. Careful necropsies were made on all the animals and the tissues sectioned and stained. The bread, rolled oats and milk diet contained approximately 65 per cent carbohydrate, 22 per cent protein, and 13 per cent fat. The lean beef heart diet contained approximately 90 per cent protein and 10 per cent fat. The rats on the 40 per cent saccharose solution consumed from 12 to 15 cc. daily.

It is seen from the tables that the animals on a diet of white bread, rolled oats and milk are much more resistant than the rats fed on a diet of lean beef heart. The resistance is markedly lowered by feeding 40 per cent saccharose solution and by fasting. For example, compare the rats that received 40 cc. 1:100 arsenic equivalent solution per kilo body weight. This represents 100 mgm. of the drug per kilo, the amount 60 per cent of the rats are required to tolerate for two days in the

TABLE 1

Arsphenamine (sodium salt) intravenously. The influence of various diets and fasting on its toxicity. Table shows the percentage of rats that died

	DOSE PER KILO OF 1:100 ARSENIC EQUIVALENT SOLUTION								
	10 cc.	20 cc.	30 cc.	35 cc.	40 cc.	50 cc.	55 cc.	60 cc.	70 cc.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Three day fast............	12 [17]	9 [23]	20 [10]		46 [24]	90 [10]			
Three days on 40 per cent saccharose solution.......		32 [28]	59 [27]		63 [43]	69 [13]			
Six days on lean beef heart.		0 [10]	0 [15]	0 [14]	42 [26]	48 [25]	50 [20]	100 [10]	
Six days on bread, rolled oats and milk...........		0 [15]	0 [13]	0 [9]	0 [15]	37 [19]	60 [20]	89 [18]	100 [10]

The numbers within the brackets represent the number of rats used in the series.

official method for testing arsphenamine. All of the rats on a diet of bread, rolled oats and milk survived and only 20 per cent when sacrificed showed kidney necrosis. The necrosis was very slight. Of the rats that received a beef heart diet, 42 per cent of the animals died, 38 per cent with the acute reaction, and 63 per cent showed kidney necrosis. As a rule, from one-fifth to two-thirds of all the kidney tubules were characteristically necrotic. Of the animals that were fasted for three days, 46 per cent died, 37 per cent with the acute reaction, and 73 per cent showed kidney necrosis. The severity of the necrosis was

TABLE 2

Arsphenamine (sodium salt) intravenously. The influence of various diets and fasting on the acute reaction. Table shows the percentage of rats that died acutely and within twelve hours after the injection

	DOSE PER KILO OF 1:100 ARSENIC EQUIVALENT SOLUTION								
	10 cc.	20 cc.	30 cc.	35 cc.	40 cc.	50 cc.	55 cc.	60 cc.	70 cc.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Three day fast.............	0 [17]	0 [23]	20 [10]		37 [24]	70 [10]			
Three days on 40 per cent saccharose solution.......		25 [28]	48 [27]		54 [43]	69 [13]			
Six days on lean beef heart		0 [10]	0 [15]	0 [14]	38 [26]	36 [25]	25 [20]	90 [10]	
Six days on bread, rolled oats and milk............		0 [15]	0 [13]	0 [9]	0 [15]	32 [19]	60 [20]	72 [18]	100 [10]

The numbers within the brackets represent the number of rats used in the series.

TABLE 3

Arsphenamine (sodium salt) intravenously. The influence of various diets and fasting on the kidney necrosis. Table shows the percentage of rats that lived twelve hours or longer and showed kidney necrosis

	DOSE PER KILO OF 1:100 ARSENIC EQUIVALENT SOLUTION							
	10 cc.	20 cc.	30 cc.	35 cc.	40 cc.	50 cc.	55 cc.	60 cc.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Three day fast..................	0 [17]	0.9 [23]	50 [8]		73 [15]	100 [3]		
Three days on 40 per cent saccharose solution................		19 [21]	93 [14]		70 [20]	100 [4]		
Six days on lean beef heart.......		0 [10]	0 [15]	50 [14]	63 [16]	88 [16]	93 [15]	100 [1]
Six days on bread, rolled oats and milk.....................		0 [15]	16 [13]	22 [9]	20 [15]	16 [13]	88 [8]	80 [5]

The numbers within the brackets represent the number of rats that lived twelve hours or longer. See table 2 for the number of rats used in each series.

about the same as that of the animals on a beef heart diet. Of the animals that received 40 per cent saccharose solution, 63 per cent of the animals died, 54 per cent with the acute reaction and 70 per cent showed kidney necrosis. The kidney necrosis was extensive and the oedema of the lungs most pronounced. In many instances the pleural cavities were filled with fluid.

We do not wish to venture a hypothesis to explain the fact that sugar feeding alone does not protect rats against arsphenamine poisoning.

b. Standardization of the rat for the biological testing of drugs

In the official method for testing arsphenamine, it is required that white rats weighing from 100 to 150 grams shall tolerate 100 mgm. of the drug per kilo body weight for forty-eight hours when given intravenously as a 2 per cent alkaline solution, 0.9 cc. of normal sodium hydrate being used for 100 mgm. of arsphenamine. It is further required that the rate of injection shall be twelve to fifteen seconds for every 0.1 cc. of solution. It is also stated in the regulations that the rats should be fed on a well-balanced ration of white bread, cracked corn, cow's milk, and, in addition, twice weekly, fresh beef and cabbage. An adequate supply of fresh, clean water should be provided at all times. The rats should have no access to food for from eighteen to twelve hours preceding the injection, though water should be supplied during this time. During the period of observation following the injection, the rats should be fed on the same mixed diet as described above.

We wish to emphasize that accurate diet control is very important in the testing of the toxicity of arsenicates. The mixed diet of white bread, rolled oats and whole milk used in our experiments was in the proportion of 1, 1, to 3. It was thoroughly mushed together in a hashing machine so that the rats were compelled to eat the mixture. The animals were fasted for seventeen hours before beginning the diet, when the initial weights were recorded. At the end of six days they were again fasted for from eighteen to twenty-two hours when the final weights were recorded. Fresh water was supplied at all times.

The final weights were used for computing the doses of the drug. Young rats weighing between 75 and 125 grams gain on the average 6 per cent during the six days. If the rats lose weight during the period it is evident that they are pathological and therefore should not be used for testing the drug.

c. The comparative toxicity of arsphenamine, neoarsphenamine and para-oxy-meta-amino-phenyl-arsenoxide

Differences in the toxicity of various arsenic preparations have been explained by Voegtlin and Smith (1920) on the hypothesis that they must be changed to one type, namely, the trivalent oxides RAsO before exerting their principle toxic action either upon protozoal parasites or upon the tissues of the host. They have pointed out that the other forms, such as arsenobenzol derivatives, $RAs = AsR$, and pentavalent arsenicals, $RAs(O)(OH)_2$, are without direct injurious action except as they are converted by oxidation or reduction, respectively, into the trivalent oxide type:

$$RAs-AsR \rightarrow RAsO \leftarrow RAs\mathord{\Big\langle}{\overset{\displaystyle OH}{\underset{\displaystyle OH}{=O}}}$$
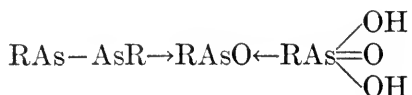
Accordingly, p-oxy-m-amino-phenyl-arsenoxide would represent the active trivalent oxide of arsphenamine. Furthermore, it is concluded that the nature and rate of oxidation of arsphenamine and neoarsphenamine to the corresponding oxides furnish an explanation of the increase in toxicity and trypanocidal activity of these compounds, when their solutions are exposed to air.

Ehrlich (1913) observed that the toxicity of a slightly alkaline aqueous solution of arsphenamine or an aqueous solution of neoarsphenamine increases rapidly when kept in contact with air.

Roth (1920) found that shaking aqueous solutions of neoarsphenamine or alkalinized arsphenamine in the presence of air increases their toxicity markedly. Shaking neoarsphenamine solution with air for ten minutes increased the toxicity over 400 per cent.

With the above facts in mind, it was decided to compare arsphenamine, neoarsphenamine and p-oxy-m-amino-phenyl-arsenoxide pathologically on a quantitative basis. The diet was controlled by placing the rats on a diet of bread, rolled oats and milk for six days before making the injections. The animals, 311 in all, were carefully necropsied and tissues sectioned and stained.

TABLE 4

Arsphenamine (sodium salt), neoarsphenamine and p-oxy-m-amino-phenyl-arsen-oxide (sodium salt) intravenously. A quantitative comparison of their toxic effect on the kidney. Rats injected intravenously after six days on bread, rolled oats and milk. Table shows the percentage of rats living twelve hours or longer and showing kidney necrosis

	DOSE PER KILO OF 1:100 ARSENIC EQUIVALENT SOLUTION														
	2 cc.	4 cc.	6 cc.	8 cc.	10 cc.	15 cc.	20 cc.	30 cc.	35 cc.	40 cc.	50 cc.	55 cc.	60 cc.	70 cc.	80 cc.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
P-oxy-m-amino-phenyl-arsen-oxide.........	0 [14]	7 [15]	38 [13]	80 [15]	100 [6]										
Neoarsphena-mine..........						0 [15]	27 [15]	21 [14]		67 [12]	87 [14]		100 [14]	90 [21]	100 [15]
Arsphenamine....							0 [15]	16 [13]	22 [9]	20 [15]	16 [13]	88 [8]	80 [5]		

The numbers within the brackets represent the number of rats that lived twelve hours or longer. See table 6 for the number of rats used in each series

Necrosis of the kidney parenchyma has been found to be the most constant lesion common to all three drugs and has been used by us to compare the toxicity of the drugs pathologically.

Table 4 shows clearly that p-oxy-m-amino-phenyl-arsenoxide is by far more toxic to the kidney than either neoarsphenamine or arsphenamine. Arsphenamine is the least toxic of the three drugs when compared on an arsenic equivalent basis. A 4 cc. 1:100 arsenic equivalent solution of p-oxy-m-amino-phenyl-arsenoxide per kilo body weight produces kidney necrosis, while it takes 20 cc. of neoarsphenamine and 30 cc. of arsphenamine. it may be said, therefore, that p-oxy-m-amino-phenyl-arsenoxide,

is five times more toxic to the rat kidney than neoarsphenamine and seven and one-half times more toxic than arsphenamine. Further, it is shown that neoarsphenamine is 50 per cent more toxic to the rat kidney than arsphenamine. It should also be emphasized that the dose of neoarsphenamine required to cause kidney necrosis in the rat on the ideal diet of bread, rolled oats and milk is only five times as large as the dose ordinarily used

TABLE 5

Arsphenamine (sodium salt), neoarsphenamine and p-oxy-m-amino-phenyl-arsen-oxide (sodium salt) intravenously. A quantitative comparison of their toxicity. Rats injected intravenously after six days on bread, rolled oats and milk. Table shows the percentage of rats that died

	DOSE PER KILO OF 1:200 ARSENIC EQUIVALENT SOLUTION*														
	2 cc.	4 cc.	6 cc.	8 cc.	10 cc.	15 cc.	20 cc.	30 cc.	35 cc.	40 cc.	50 cc.	55 cc.	60 cc.	70 cc.	80 cc.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
P-oxy-m-amino-phenyl-arsen-oxide.........	0 [14]	0 [15[	8 [13]	7 [15]	45 [11]										
Neoarsphena-mine..........						0 [15[	0 [15]	7 [15]		8 [13]	7 [15]		7 [15]	14 [21]	7 [15]
Arsphenamine....mine..........							0 [15]	0 [13]	0 [9]	0 [15]	37 [19]	60 [20]	89 [18]	100 [10]	

* 10 cc. 1:100 arsenic equivalent solution contained either 26.78 mgm. p-oxy-m-amino-phenyl-arsenoxide, 39.33 mgm. neoarsphenamine or 24.88 mgm. arsphenamine. The numbers within the brackets represent the number of rats used in the series.

clinically, while the dose of arsphenamine required is almost eight times as large.

Table 5 gives a clear cut picture of the death rate of animals on a constant diet. Roth (1920) compared the toxicity of arsphenamine and neoarsphenamine on about 1400 rats. He observed that approximately all the deaths due to arsphenamine occur within two days after the injection and those from neo-arsphenamine within five days In order to study the pathology of the tissues the arsphenamine and p-oxy-m-amino-phenyl-arsenoxide rats were sacrificed after two days and the neo-arsphenamine rats were sacrificed after five days.

Table 6 shows the percentage of rats that died acutely. Usually the acute deaths following the injection of arsphenamine and p-oxy-m-amino-phenyl-arsenoxide were characterized by distention, congestion, oedema and petechiae of the lungs with dilatation of the right heart and congestion of the abdominal viscera.   The acute deaths following p-oxy-m-amino-phenyl-arsenoxide were also invariably accompanied by a marked increase in the pleural fluid.   Following the intravenous injection

TABLE 6

Arsphenamine (sodium salt), neoarsphenamine and p-oxy-m-amino-phenyl-arsen-oxide (sodium salt) intravenously. A quantitative comparison of the acute reactions. Rats injected intravenously after six days on bread, rolled oats and milk. Table shows the percentage of rats that died acutely and within twelve hours after the injection

	DOSE PER KILO OF 1:100 ARSENIC EQUIVALENT SOLUTION														
	2 cc.	4 cc.	6 cc.	8 cc.	10 cc	15 cc.	20 cc.	30 cc.	35 cc.	40 cc.	50 cc.	55 cc.	60 cc.	70 cc.	80 cc.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
P-oxy-m-amino-phenyl-arsen-oxide.........	0 [14]	0 [15]	0 [13]	0 [15]	45 [11]										
Neoarsphenamine..........						0 [15]	0 [15]	7 [15]		8 [13]	7 [15]	·	7 [15]	0 [21]	0 [15]
Arsphenamine....							0 [15]	0 [13]	0 [9]	0 [15]	32 [19]	60 [20]	72 [18]	100 [10]	

The numbers within the brackets represent the number of rats used in the series.

of neoarsphenamine the acute deaths were associated with severe bleeding from the site of injection or with other inexplicable accidents.   There was no distention or oedema of the lungs and the pleural fluid was not increased.   These results are in harmony with those of Jackson and Smith (1918).   These authors conclusively demonstrated by pharmacological experiments on dogs that either acid or alkaline solutions of arsphenamine given intravenously in toxic doses produce a very marked and prolonged rise in the pulmonary blood-pressure, a rise that may be 100 per cent above the normal.   In a later paper by Smith (1920)

it was shown that p-oxy-m-amino-phenyl-arsenoxide affected the pulmonary blood pressure in a manner quite comparable with that of a solution of arsphenamine of corresponding strength. Neoarsphenamine, in the same concentration and the same rate of injection was without effect on the pulmonary blood pressure. Jackson and Raap (192) have further shown that good preparations of arsphenamine have almost no direct action on the bronchial musculature of the dog.

In conclusion, it should be emphasized that the dose of arsphenamine required to produce the acute lung picture with death in rats on a bread, rolled oats and milk diet is twelve times greater than the dose used clinically.

d. Comparison of kidney lesions produced by arsphenamine, neo-arsphenamine and para-oxy-meta-amino-phenyl-arsenoxide

In general the kidney lesions produced by arsphenamine and neoarsphenamine in rats agree with those described by Pearce and Brown (1915) for dogs and guinea-pigs. We have found the lesions produced by p-oxy-m-amino-phenyl-arsenoxide to be of the same general type.

The kidneys of rats that are killed acutely with arsphenamine or p-oxy-m-amino-phenyl-arsenoxide are usually red. Microscopically, the blood vessels are engorged and interstitial hemorrhages are frequent in the boundary zone of the medulla. The kidneys of rats that die after twelve to twenty-four hours are swollen and show opaque, yellowish white striations representing tubular necrosis. Microscopically, the essential feature is the tubular necrosis. After a large dose of the drug from one-third to two-thirds of all the kidney tubules may contain necrotic parenchyma cells. The cells are swollen, desquamated and appear as pink-staining hyaline masses. The nuclei have either disappeared or remain as pycnotic, deeply stained bodies. In many of the tubules the entire epithelium may be destroyed and appear as homogeneous pink-staining slightly granular masses. The distribution of the tubules containing necrotic cells may be very irregular. Tubules in the outer cortex, inner cortex and the boundary zone of the medulla may be affected. For p-oxy-

m-amino-phenyl-arsenoxide the most frequent site is the inner cortex and boundary zone of the medulla. Usually the glomeruli are large. The tufts well fill the capsular spaces and do not appear especially abnormal. Oedema of the labyrinth occurs infrequently. The collecting tubules frequently contain casts.

The changes produced in the kidney by neoarsphenamine are far more severe when compared with those produced by the same arsenic equivalent dose of arsphenamine. In gross, the kidneys are enlarged, swollen, opaque and pale. Microscopically, the tubular necrosis is the most characteristic feature. Calcification of the necrotic cells is frequent. Here again the distribution of the necrosis may be very irregular affecting tubules in any portion of the cortex or the tubules in the boundary zone of the medulla. The glomeruli are usually large. The tufts may well fill the capsular spaces or they may be small and appear compressed by an abundant albuminous precipitate in the capsular spaces. The tuft vessels usually contain endothelial cells and an increased number of polymorphonuclear leucocytes within their lumina. The collecting tubules contain casts.

EXPERIMENTS WITH DOGS

*a. Kidney lesions produced by arsphenamine, neoarsphenamine.
The influence of mercury*

These experiments were undertaken by Dr. Kolls in order to study the influence of various doses of arsphenamine upon the function of the kidneys. The disodium salt of the drug was used for the major portion of the work but the monosodium salt, the dihydrochloride as well as neoarsphenamine were also employed. The dog was chosen as the most desirable species for the experiments. Observations were continued over a considerable period in order to determine the presence or absence of chronic lesions. It was deemed probable that, if conditions could be found under which a degree of renal insufficiency could be produced at will, even if it were temporary, a means would be at hand for the study of the effect of various prophylactic and therapeutic measures upon the course of the lesion. It

was also thought important to correlate the observations made with the actual histology of the organ and to this end the animals were necropsied and the organs studied pathologically. Finally an attempt was made to determine what influence the administration of mercury in various forms and by various routes would have on the course of the lesion. The detailed report of the urine and blood findings will be published later.

In brief it may be said that all the animals receiving 50 mgm. disodium arsphenamine per kilo survived. This dose invariably caused albuminuria, and the appearance of casts in most instances. The function of the kidneys as judged by the reduction in quantity, the excretion of urea, creatinine and phenolsulphonephthalein was not always impaired, the same animal reacting quite differently to the same dose when repeated. With the exception of one, all animals receiving 75 to 100 mgm. per kilo died. Where the kidney function was depressed it remained so for only a few days. In no case was the secretion completely suppressed. The doses that proved fatal did not cause any grave immediate change in renal efficiency. The late symptoms of the intoxication are those of profound shock, subnormal temperature, cyanosis, rapid and shallow respiration, low blood pressure, and feeble pulse. Four of the animals developed definite icterus accompanied by extensive central hyaline liver necrosis.

The recoveries from sublethal doses are apparently permanent. Chronic nephritis was not produced in a single instance. The excretion of phenolsulphonephthalein was normal and the urea of the blood remained at the original level. Albuminuria often persisted but was due, in all probability to the cystitis set up by the daily catheterization. Occasionally a few hyaline casts were seen in the centrifuged specimens. These are of little significance in the urine of the dog. Evans, Wynne and Whipple (1912) have conclusively demonstrated that catheterization of the dog's bladder is followed by a reflex albuminuria and hyaline casts.

The two animals that received 100 mgm. neoarsphenamine per kilo and were sacrificed after three weeks showed a surprising

amount of renal disturbance. In both instances a prolonged renal depression followed and at necropsy the kidneys showed subacute diffuse nephritis.

The administration of mercury in doses corresponding to therapeutic doses in man apparently does not materially increase the severity of the renal injury produced by arsphenamine. Furthermore, the kidney injury produced by toxic doses of mercuric chloride is not greatly influenced by arsphenamine.

b. Liver necrosis with jaundice

As stated above four of the dogs receiving disodium arsphenamine intravenously developed definite jaundice accompanied by extensive central liver necrosis. The dogs received a diet of medium fat beef preceding the administration of the arsphenamine. They were given a single dose of from 65 to 100 mgm. per kilo and died either on the third or the fifth day following the injection. The necropsies showed extensive central liver necrosis, in some instances comparable with acute yellow atrophy. The livers were swollen and quite fatty in appearance. The capsules were thin. On cross section the lobules were sharply outlined with deep red centers and opaque yellowish-brown peripheries. The gall bladder and bile ducts were normal. Microscopically there was an extensive central necrosis. From one-third to four-fifths of the liver cells surrounding the efferent veins were killed and appeared as pink-staining hyaline masses. These areas contained a great many wandering cells and red blood cells. The liver cells about the portal areas were swollen and contained many fat droplets (fat stain, osmic acid). The bile ducts were unchanged.

c. Mechanism of the jaundice following the administration of arsphenamine and neoarsphenamine

We do not wish to enter upon the consideration as to whether the jaundice following the administration of arsphenamine or neoarsphenamine to syphilitic patients is due to the disease or to the drug. We submit experiments on dogs which show conclusively that arsphenamine itself is capable of causing jaundice

with liver necrosis in some instances comparable with acute yellow atrophy. The type of necrosis is essentially the same as that described for chloroform poisoning by Whipple and Sperry (1909), except that the necrotic areas contain a greater number of red blood cells.

GENERAL SUMMARY

The pathology of experimental arsphenamine, neoarsphenamine and p-oxy-m-amino-phenyl-arsenoxide poisoning is described on a quantitative basis.

Starved animals and those fed sugar alone in the days preceding the intravenous administration of sodium arsphenamine are very susceptible to arsphenamine poisoning. A maximal kidney injury is to be expected.

A diet of white bread, rolled oats and whole milk greatly increases the resistance and exerts a marked protective action against the kidney injury.

A method is proposed for the standardization of the rat for the biological testing of drugs. Accurate diet control in the days preceding the administration of the drug is very important. Animals unfit for the test may be detected by following the weight curves.

Neoarsphenamine is much more toxic to the rat and dog kidney than sodium arsphenamine. The kidney injury produced by arsphenamine is transient while that caused by neoarsphenamine may be permanent.

Para-oxy-meta-amino-phenyl-arsenoxide has been found to be five times more toxic to the rat kidney than neoarsphenamine when compared on an arsenic equivalent basis and seven and one-half times more toxic than sodium arsphenamine.

Administration of mercury in doses corresponding to therapeutic doses in man apparently does not materially increase the severity of the renal injury produced by arsphenamine.

Lethal doses of sodium arsphenamine administered intravenously to dogs on a diet of medium fat beef may be followed by jaundice and liver necrosis in some instances comparable with acute yellow atrophy.

Arsphenamine and neoarsphenamine react differently in the animal organism and should not be considered as one and the same compound.

Good preparations of arsphenamine and neoarsphenamine when administered in doses corresponding to those ordinarily used clinically are harmless to the normal animal organism.

The detailed report will appear in the near future as a Hygienic Laboratory Bulletin.

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EFFECTS OF VASOMOTOR DEPRESSANTS UPON THE VOLUME OF THE LIVER

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Some time ago I carried out a series of experiments upon volume changes in the liver (1), especially as they are affected by the injection of epinephrine, and I found among other things that the main vasomotor changes came through the hepatic artery, the portal circulation playing a very minor rôle. Further, that when the hepatic artery is clamped, the changes in volume which follow upon epinephrine administration, are due mainly to pressure changes in the vena cava and are, therefore, passive in character. I plan to discuss this phase of the question again in a later paper, as other workers have recently published results upon the same subject which are not in entire harmony with those in my communication.

At this time, however, I wish to report upon some observations which I have carried out upon the changes in the liver circulation which are produced by drugs whose characteristic action is a lowering of blood pressure, such as the nitrite series. In view of the interesting changes produced by the vasoconstrictors and the bearing which their effects had upon our knowledge of vasomotor control in the portal area, it was thought that an examination of the effects of the vasodilators would not be without interest and might add something to our knowledge of the same subject.

One of the earlier workers who investigated the circulatory changes in the liver as the result of amyl nitrite inhalation was Burton-Opitz (2) who found that, when measured with his stromuhr, the arterial inflow was decreased markedly as soon as

the drug had exercised its characteristic effect upon the systemic blood pressure. This decrease in arterial inflow he ascribes to loss of driving force due to relaxation of the vascular channels.

Reid Hunt (3), in his extensive series of experiments with acetyl-cholin reports not only the effect of this drug upon the volume of the liver, but also reports some experiments with the nitrites. These results may be discussed together, as in his experiment 481 (fig. 2) upon a dog, he shows a decrease in liver volume resulting from acetyl-cholin and states that nitroglycerin produced almost identical results. In a cat experiment 488 (fig. 3), he found that with the coeliac axis tied, the cholin compound had no effect upon the liver volume.

In general, he found that acetyl-cholin produced a diminution in the volume of the organ which was probably passive, being due to the lowered blood pressure resulting in a lessened blood supply to the organ through the hepatic artery, as it did not occur when that vessel was clamped. He believes that the drug has a dilator action upon the terminations of the hepatic artery, but that this is obscured by a greater vasodilation elsewhere. Hunt also found in the cat that acetyl-cholin caused a fall in portal blood pressure probably due to lessened outflow from the intestinal veins. These findings with acetyl-cholin are interesting, as they are in close harmony with results which I have observed with the nitrites except as to the effect when the hepatic artery is clamped.

My own experiments were carried out in the same manner as I described earlier in my paper on the "Vasomotor reactions of the liver" (1). The dogs were anesthetized with morphine and chloretone; tracheal, arterial and venous cannulae inserted and the liver oncometer adjusted in the usual manner. The amyl nitrite was administered by dropping the required dose into the side arm of the tracheal tube. The results showed that the effect of nitrites upon the liver volume was apparently comparatively simple when compared with the complex changes which followed upon the administration of epinephrine. Immediately following the fall in systemic blood pressure which resulted from the giving of amyl nitrite in from 3 to 5 drop doses the volume of the liver

decreased and this decrease began in from five to ten seconds
after the blood pressure decline had begun.   Also in contrast
with the results obtained with epinephrine the clamping of the
hepatic artery had little effect so far as the general course of
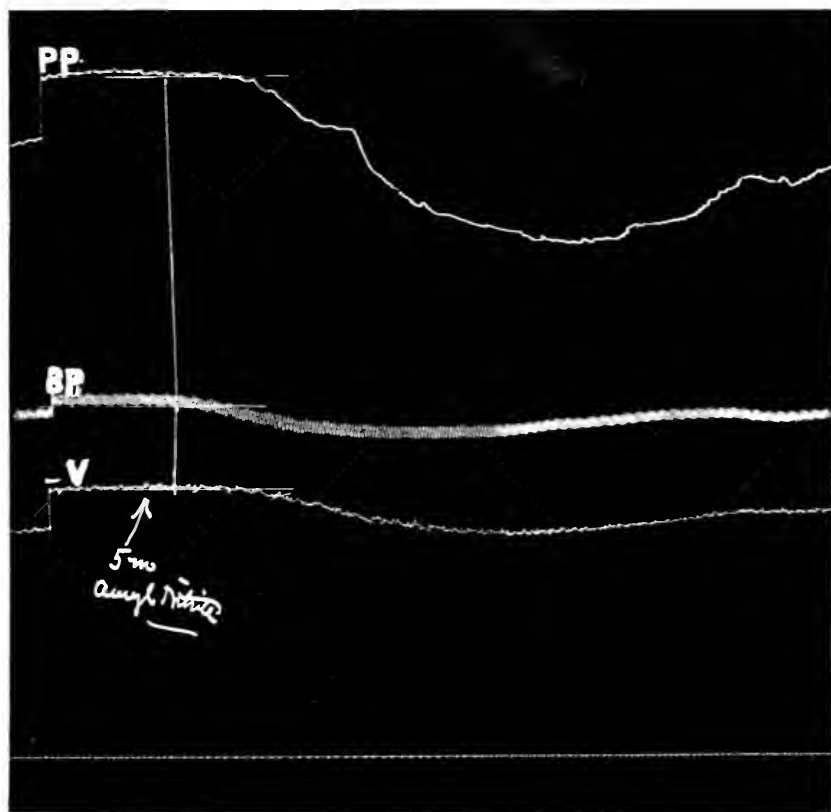
FIG. 1.   AMYL NITRITE EFFECT UPON PORTAL PRESSURE AND LIVER VOLUME
IN THE DOG

Hepatic artery is clamped.   B.P., blood pressure; P.P., portal blood pressure;
L.V., liver volume.   Time in seconds.

events was concerned (fig. 1), but in some experiments the de-
crease in volume came on distinctly earlier than it had done
when the arterial circulation was intact.   This may have been
accidental in those cases in which it occurred as it was not uni-

formly present. These findings are not in harmony with the observation of Hunt, who reported that in a cat, clamping the coeliac axis while it did not prevent a marked fall in systemic blood pressure from acetyl-cholin, yet it did prevent the change in liver volume when the pressure fell.

The relation of the portal pressure to the liver volume decline was important especially as regards its time relations as it was found that it too suffered a marked decrease when the nitrite was given. In some cases both portal pressure and liver volume declined simultaneously but in certain other observations the liver volume decreased distinctly before the portal pressure fell and it was found that this difference was clearest and most distinct when the hepatic artery was open. This difference is easily explained by the fact that with the direct blood channel open there would be an immediate effect due to the lowered systemic blood pressure.

In a number of animals the vena cava pressure was measured at the same time as the liver volume changes were registered, but in no case was there any considerable change found which could have influenced in any marked way the liver volume. In every experiment the caval pressure fell below normal. When the hepatic artery was open, this fall came early, practically at the same moment as the organ volume decreased while if the artery was closed, the decrease in pressure came rather later. The changes in caval pressure under amyl nitrite therefore, were not great and they seemed to be due entirely to, or at least to accompany, the changes in circulation reflected in the liver volume changes. This was also in marked contrast to the condition when epinephrine was given, in which case with the hepatic arterial circulation shut off the changes in pressure in the cava greatly influenced the volume of the liver.

The comparative inactivity of the liver vessels to nitrites was demonstrated in an experiment in which the stomach, intestines and spleen were removed from the body, the hepatic artery being left intact. A paraffined cannula pointing centrally was inserted into the abdominal aorta and a second cannula into the portal vein pointing toward the liver. These two can-

nulae were connected by paraffined rubber tubing. Tracings could now be taken without the interference caused by the intestinal vessels. The findings confirmed those obtained in the experiments on the intact animal. In all cases the fall in general blood pressure was followed in a few seconds by a decline in the liver volume, no matter whether the organ received its blood through the hepatic or through the portal, or through both simultaneously. The decline in volume was, on the whole, a little more prompt in making its appearance when both channels were open, which was only to be expected.

The general conclusion to be drawn from these experiments regarding the effects of the nitrites upon the blood supply of the liver (and indeed upon the portal blood pressure) is that it is purely an indirect one, due to changes induced in the systemic circulation which bring about a lessened blood supply to the organ. There was no evidence found of any active dilatation in either the portal or in the hepatic vessels. It is possible that the hepatic artery may have been relaxed and yet not show it by a change in liver volume, but my results harmonize well with those of Burton-Opitz, who as stated, found a lessened arterial inflow due to nitrite.

EFFECT OF THE DEPRESSANT SUBSTANCE IN DOG'S URINE UPON LIVER VOLUME

Another agent which is very powerful in its ability to lower blood pressure is the depressor substance to be found in dog's urine. This substance has been studied by Pearce and Eisenbrey (4), who reported that coincident with the marked but transient decrease in general blood pressure, there was a decrease in the size of the kidney, spleen and intestine, the brain and leg undergoing a similar, but less marked change. The pressure in the vena cava underwent a barely perceptible increase.

In connection with my work upon the effect of nitrite upon the volume of the liver, I also carried out a number of experiments with dogs' urine, for the purpose of comparison with the nitrite effects. I injected the urine in 3 cc. and 4 cc. doses and found the same changes in general blood pressure and in intestinal

volume as were described by Pearce and Eisenbrey, but together with the decline in intestinal volume, I found also a simultaneous decrease in liver volume following a transient increase, the two going practically parallel. The pressure changes in the vena
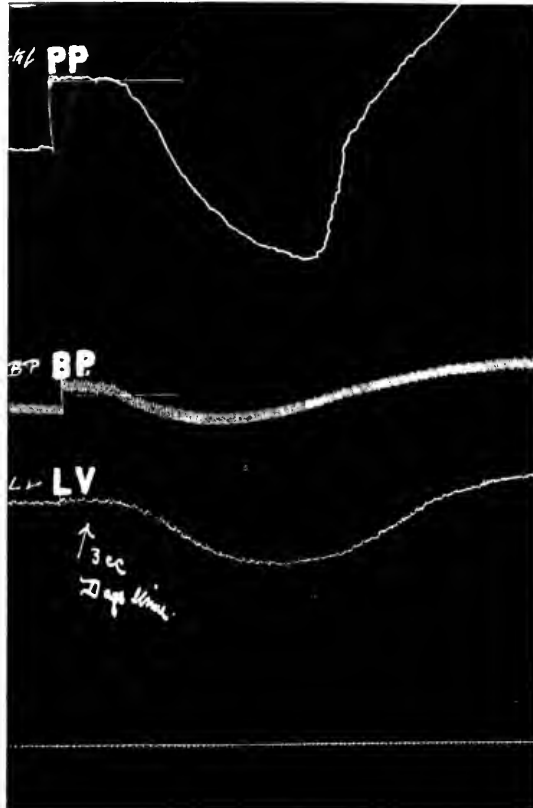
FIG. 2. EFFECT OF THE INJECTION OF 3 CC. OF DOG'S URINE UPON THE PORTAL
BLOOD PRESSURE AND LIVER VOLUME IN THE DOG

Hepatic artery clamped. *P.P.*, portal blood pressure; *L.V.*, liver volume; *B.P.*, blood pressure. Time in seconds.

cava and in the portal vein were also investigated. In the former I found, as did Pearce and Eisenbrey, that there was a very slight but hardly perceptible increase in pressure which had no time relation to the liver volume changes as it lasted not only during

the time when the liver showed its preliminary increase, but also for a considerable part of the interval during which the liver volume was below normal. It clearly could not have played any part in the change in volume which the liver underwent.

The relation of the portal pressure to the decline in the volume of the liver was harder to estimate as in certain experiments it was found that the liver volume fell before the portal pressure underwent any decline. However, this interval, which was very slight (not more than two or three seconds), disappeared when the hepatic artery was clamped, after which portal pressure and liver volume decreased simultaneously as nearly as could be determined. The early decline in liver volume was therefore due to the lessened volume of blood which came to the organ through the hepatic artery when the systemic pressure was lowered. On the other hand, the main decrease in volume was due to the lessened supply of blood which the organ received through the portal vein from the organs which that vessel drains.

An interesting point which is raised by these results is as to what becomes of the blood when the systemic pressure is lowered by the depressor substance in dogs' urine. It is clearly not in the spleen, kidneys, intestines, or muscles, according to the findings of Pearce and Eisenbrey nor is it in the liver as all these organs show a decrease in volume. The question arises as to whether it is not in the vena cava but if so that vessel must have undergone a very marked dilatation, otherwise the pressure in it would have been increased very considerably, which has been shown not to take place.

A question closely connected with the foregoing is the relation of the vessels of the different abdominal organs to the distribution of the blood under the various vasomotor depressants. With the nitrites which act upon non-striated muscle the intestinal vessels dilate and receive the blood at the expense of the liver. This distribution of the blood may be explained by the greater amount of muscular tissue available in the vessels which drain into the portal vein.

With the depressant substance in dogs' urine which is said to act upon the nerve endings in the vessel walls, the organs

supplying the portal vein and the liver itself are all depleted, undergoing a marked diminution in volume, the blood accumulating possibly in the main venous trunks of the body. The powerful vasodepressant, acetylcholin, was found by Hunt to act upon a vasodilator mechanism which was different from that involved in the action of any nerves to which a vasodilator action had previously been ascribed. The most marked dilator action seemed to be in the vessels of the skin. The vessels of some of the abdominal organs were also involved in the dilatation. In histamine shock according to Dale and Laidlaw (5), the fall in blood pressure is due to capillary relaxation, the blood accumulating in the venules and capillaries. There is no increase in liver volume and the portal vein is flat and collapsed. In anaphylactic shock in dogs (6), peptone intoxication (Thomson (7)) and with the split protein poison of Vaughan (Edmunds (8)) there is also a very marked fall in systemic blood pressure but in these cases, the liver receives the blood at the expense of the other abdominal organs. In the cases of these poisons, as in the urinary depressant substance discussed, the effect is said to be upon the nerve endings but with very different results ensuing, so far as the ultimate distribution of the blood is concerned.

SUMMARY

The nitrite group of vaso-motor depressants lowers the portal blood pressure and decreases the volume of the liver. These effects are secondary to the changes produced in the general systemic blood pressure. The blood tends to accumulate therefore in other abdominal organs than the liver in contrast to the condition in anaphylactic shock in dogs and also as a result of certain poisons where the liver drains the remaining abdominal organs.

In the case of still other vasodilators, such as the depressant substance in dogs' urine, the exact location of the blood when the action is fully developed is not accurately known. This point together with an effort to bring some of these facts into an harmonious whole upon some definite physiological basis is a subject requiring further investigation.

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THE RELATION OF THE DEXTROSE AND WATER CONTENT OF THE BLOOD TO ANTIPYRETIC DRUG ACTION[1]

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The antipyretic action of dextrose was pointed out by one of us(1) two years ago. It occurs when the sugar is given by mouth or intravenously to peptone-fever rabbits and has also been studied in some detail in fever patients, particularly cases of tuberculosis. In the rather large literature which has accumulated about the clinical employment of sugars, owing to the recent investigations of shock by Erlanger and Woodyatt (2) and of tuberculosis by Lo Monaco (3) and his school, antipyretic effects have received slight attention. Litchfield (4), however, observed them frequently after dextrose in pneumonia, typhoid, and other infections.

The effects of intravenous injections of dextrose in coli fever dogs have been investigated by Barbour and Howard (5) who showed that the antipyretic action is paralleled by a decrease in the blood solids, that is, it is exhibited during the temporary stage of blood dilution. From 2 to 4 grams per kilo were given in 50 per cent solution. In normal dogs the same injection produced much less hemodilution and correspondingly no fall in body temperature. In both normal and fever dogs was found by the end of the first hour after injection a tendency toward a high blood concentration and a rise in temperature, which

phenomenon is well known as Woodyatt's sugar dehydration fever (6).

The fact that the antipyretic phase of dextrose action is exhibited especially in the febrile and not in the normal state, which is readily explained by the presence of more available water in the tissues in the former condition, led to the hypothesis that the action of antipyretic drugs can be explained along similar lines.

The present work was therefore undertaken to determine what effect common types of antipyretic drugs have upon the dextrose and the water content of the blood in febrile as well as non-febrile conditions, and, if possible to bring this into relation with their characteristic action. The drugs to which our studies have thus far been limited are sodium salicylate, acetyl-salicylic acid, antipyrine, and quinine. (The last mentioned is not now regarded as a reliable "antipyretic" drug in ordinary therapeutic doses.)

LITERATURE

Antipyretics and carbohydrate metabolism. According to Lepine and Porteret (7) and to Nebelthau (8), antipyrine and acetanilid are capable of promoting the storage of glycogen in both liver and muscles. Noorden (9) examined the claim that salicylates decrease the sugar output in diabetes and failed to establish it. Starkenstein (10) claimed that such drugs prevent the mobilization of liver glycogen by epinephrin because the epinephrin glycosuria was found diminished or absent. But Mansfeld's (11) work shows that this is probably a renal effect for he could demonstrate no effect of antipyretic drugs upon the somewhat variable curve of epinephrin hyperglycemia. His investigations upon the blood sugar content were however not extended to the uncomplicated effect of the antipyretic drugs themselves.

We find but two cases in the literature where this has been done: Wacker and Poly (12) have described a rise in the blood sugar content in a few observations upon rabbits and tuberculous patients after phenacetine, and Silberstein (13) found that quinine in large doses produces hyperglycemia in dogs.

Antipyretics and water metabolism. The above mentioned work of Mansfeld indicates the tendency of antipyretic drugs to cause anuria. Hanzlik, Scott and Reycraft (14) have described anuria from the thera-

peutic use of salicylates.   Furthermore Hirschfeld (15) maintains that
antipyretics relieve diabetes insipidus, and Gaulier (16) finds that
salicylates diminish the excretion of chlorides in the urine.

The general tendency of these drugs to diminish urine flow might con-
tribute toward keeping the blood in a state of dilution.   No definite
changes in blood concentration resulting from antipyretic drugs have
been described.   In Hanzlik's non-febrile patients the hemoglobin con-
centration was found unaltered by salicylates, thus indicating a constant
water content of the blood.

METHOD

For this work as for other heat regulation studies, we have
selected mongrel dogs approximating 10 kilos in weight, which
were neither long-haired nor excessively short-haired.   These
dogs were kept usually for one or more weeks upon a constant
diet of meat, bread, and lard, and were fasted before each ex-
periment for a period of at least twenty-four hours.

For *blood sugar* determinations the method of Benedict (17) was em-
ployed in the majority of cases, subject to the following slight modi-
fication: instead of using a measured amount of oxalated blood, fifteen
or sixteen drops of blood freely flowing from the ear vessels were
collected into 4 c.c. of water in a weighing bottle, the exact amount of
blood being determined gravimetrically.   The blood water mixture was
then made up to 12.5 c.c. with picric acid-picrate solution and filtered
in the usual manner.

The colorimetric method of Folin and Wu (18) was employed simul-
taneously with the Benedict method in a number of experiments.

The blood concentration in the majority of cases was determined by
the hemoglobin method of Cohen and Smith (19), while in a number of
experiments determination of the blood solids percentage (in 15- and 16-
drop samples) was substituted.

In each experiment the drug was given after two or three
control determinations of body temperature with simultaneous
withdrawal of blood samples for sugar and water determinations.
All of the antipyretics used were injected subcutaneously with
the exception of acetyl-salicylic acid, which was given per os.
Determinations of temperature and of sugar and water content

of the blood were made at hourly or shorter intervals after the injection; the first sample was usually taken within one-half hour.

Particular care was taken to avoid procedures which might excite the animal before or during the period of experimentation.

Fever was produced when desired by the injection of 0.1 to 0.5 cc. per kilo of a coli vaccine. In the dogs of the "H "series the vaccine (for help in the preparation of which we are greatly indebted to Dr. George H. Smith) contained 325,000 million killed colon bacilli per cubic centimeter. In the other experiments was employed a special preparation of Lederle containing 1,000,000 million killed coli per cubic centimeter. In most cases coli injection was made about sixteen hours before the experiment but when this delay was allowed it was found advisable to inject at least two dogs from which the one showing the highest abnormal temperature on the following morning could be selected. If on the other hand it was elected to give the antipyretic drug at the height of the coli fever, that is three or four hours after injection, the effect of the bacterial toxin was often found so strong as to preclude a marked antipyretic response to the drug.

<div align="center">RESULTS</div>

The respective effects of sodium salicylate, antipyrine, and quinine upon the *dextrose concentration of the blood* in dogs, as determined by the Benedict method, are illustrated by figures 1 to 6 (the short dash lines). In the case of each drug a pair of experiments is represented of which one is upon a normal dog and the other upon a coli fever dog, as indicated in the figures. While the blood sugar curve is more or less characteristic for each drug, other experiments in each series have shown that it is not safe to lay emphasis upon this point. The changes while definite are not of great magnitude, the concentration rarely rising above 0.25 per cent.

The *temperature* changes in these experiments are illustrated by the light continous line and the typical effect of pronounced antipyretic action is seen in the coli fever dogs, while in the
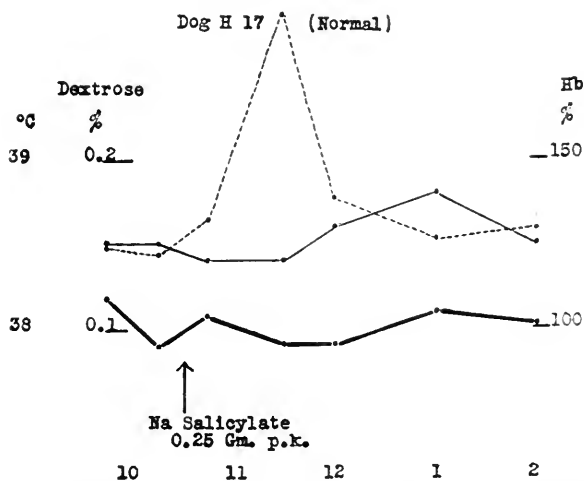
FIG. 1. EXPERIMENT H17. EFFECT OF SODIUM SALICYLATE ON NORMAL DOG

Rectal temperature: light line.   Blood dextrose per cent: broken line.   Hemoglobin per cent of standard: heavy line.
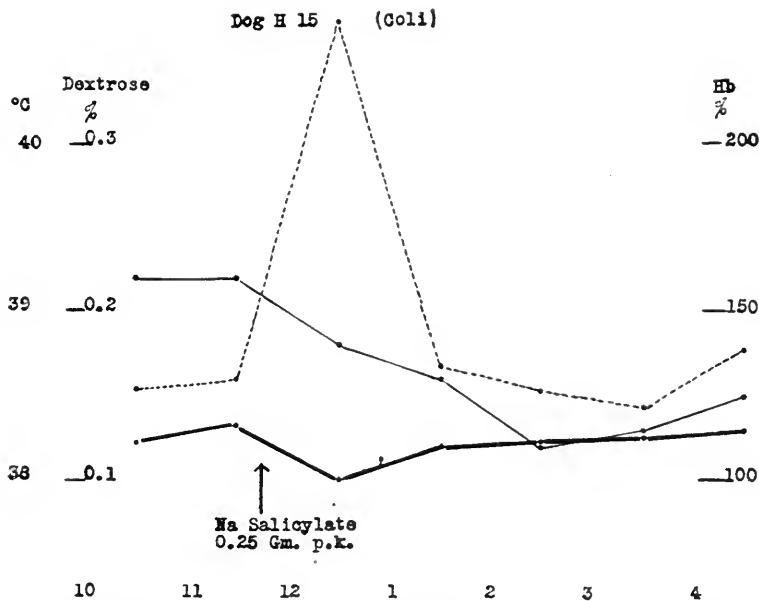
FIG. 2. EXPERIMENT H15. EFFECT OF SODIUM SALICYLATE ON COLI FEVER DOG

Rectal temperature: light line.   Flood dextrose per cent: broken line.   Hemoglobin per cent of standard: heavy line.

Dog H 25 (Normal)
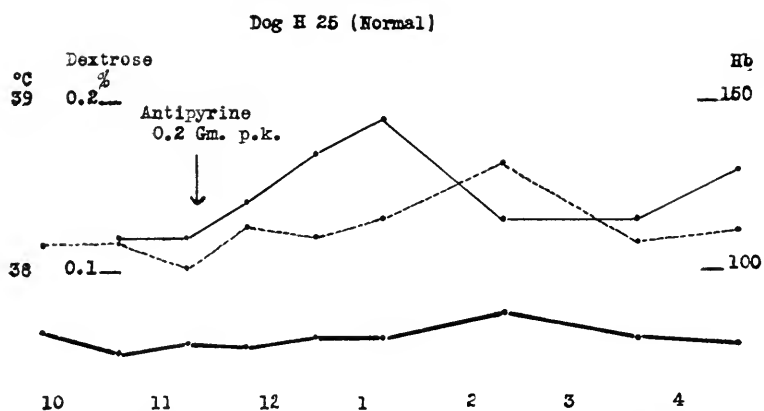
FIG. 3. EXPERIMENT H25. EFFECT OF ANTIPYRINE ON NORMAL DOG

Rectal temperature: light line.　Blood dextrose per cent: broken line.　Hemoglobin per cent of standard: heavy line.

Dog H 33 (Coli)
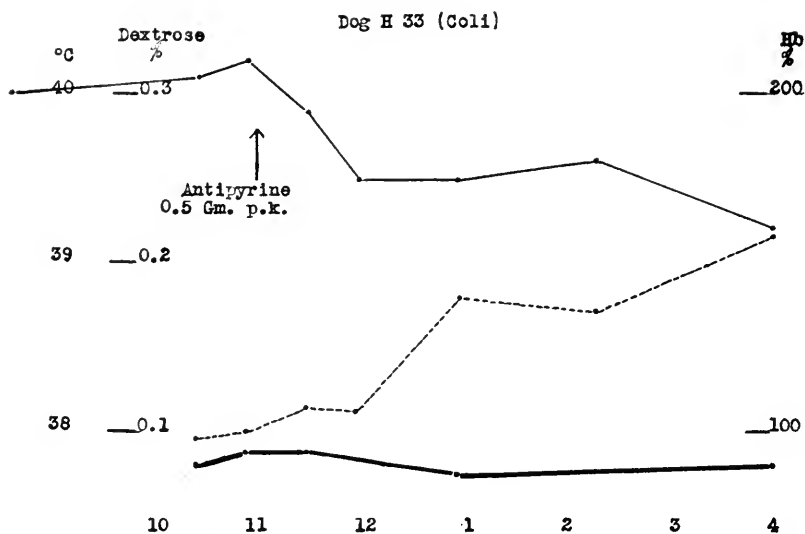
FIG. 4. EXPERIMENT H33.　EFFECT OF ANTIPYRINE ON COLI FEVER DOG

Rectal temperature: light line.　Blood dextrose per cent: broken line.　Hemoglobin per cent of standard: heavy line.
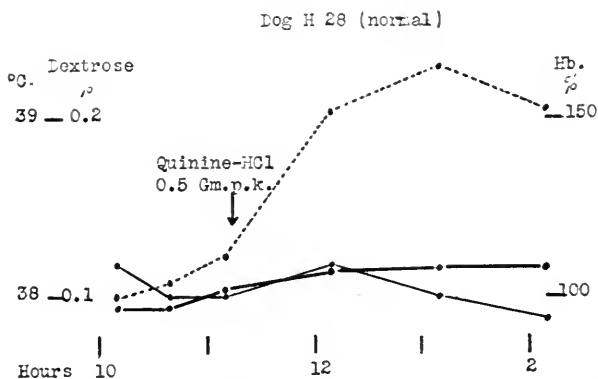
FIG. 5. EXPERIMENT H2S. EFFECT OF QUININE-HYDROCHLORID ON NORMAL DOG

Rectal temperature: light line. Blood dextrose per cent: broken line. Hemoglobin per cent of normal: heavy line.
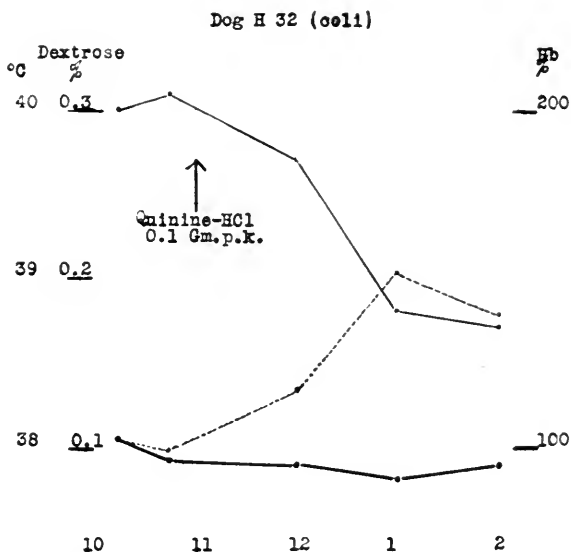
FIG. 6. EXPERIMENT H32. EFFECT OF QUININE-HYDROCHLORID ON COLI FEVER DOG

Rectal temperature: light line. Blood dextrose per cent: broken line. Hemoglobin per cent of normal: heavy line.

normal dogs the tendency is for the drug to increase oftener than decrease the temperature.

The changes in the *hemoglobin* concentration show in general a parallelism with the temperature changes. In the fever dogs the blood becomes diluted, thus accounting for the antipyretic action; in the normal dogs the hemoglobin may be somewhat variable or may rise in a similar way to the body temperature curve. In our preliminary paper were shown two figures illustrating similar effects from acetyl-salicylic acid given by mouth respectively to normal and fevered dogs.
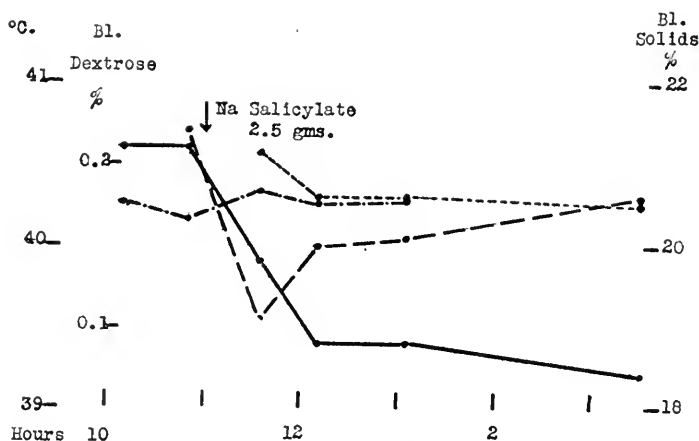
Fig. 7. Experiment 60. Effect of Sodium Salicylate on Coli Fever Dog

Rectal temperature: heavy line. Blood dextrose per cent: Benedict method, short dash line. Folin and Wu method, dot and dash line. Blood solids per cent: long dash line.

Demonstration of the blood sugar increase by the method of Folin and Wu. As an additional check upon the increase in blood sugar concentration produced by these drugs the Benedict method was controlled by simultaneous determinations with the Folin and Wu method in a number of experiments, chiefly with sodium salicylate. While the readings given by the two methods often showed a very satisfactory degree of correspondence, in cases where they differed essentially the Benedict readings were always considerably higher than the others.

We are therefore compelled to believe that in addition to the increase in blood sugar, salicylates evoke in the blood an excess of other color yielding substances. Possibly creatin or creatinin are the responsible factors. In figures 7 and 8 will be seen the results of two such experiments, the Benedict determinations being connected by the usual short dash lines, and the Folin and Wu determinations by dashes alternating with dots. In both these experiments the salicylate increased the reducing substances in the blood but many of the Benedict readings are higher; in figure 7, however, the second and third Benedict readings correspond closely to the Folin and Wu determinations and the same is true of the first and third readings in figure 8.
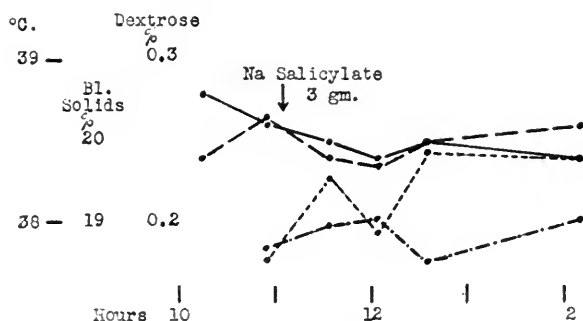
FIG. 8. EXPERIMENT 57. EFFECT OF SODIUM SALICYLATE ON COLI FEVER DOG

Rectal temperature: heavy line. Blood dextrose per cent: Benedict method, short dash line. Folin and Wu method, dot and dash line. Blood solids per cent: long dash line.

Dextrose concentration and total blood dextrose. Accepting either the hemoglobin or total solids determinations as indicators of the fluid content of the blood, one may readily calculate the relative percentage changes produced by antipyretic drugs in the total blood dextrose. This is illustrated in tables 1 and 2 in which the effects of the drugs upon blood dextrose have been compiled.

Normal dogs (summary). Table 1 illustrates the effects of the four drugs upon a series of sixteen normal dogs. In the third column are seen the doses in grams per kilo given in each

experiment and in the fourth column the maximum effect upon the body temperature, which averages a few tenths above the normal readings. Pre-injection averages of the blood deter-

TABLE 1

Dextrose in blood of normal dogs

LOG NUMBER	DRUG	PER KILO DOSE	BODY TEMPERATURE, MAXIMUM EFFECT	AVERAGE BEFORE DRUG			MAXIMUM AFTER DRUG			
				Dextrose concentration	Dextrose/Hb.	Dextrose/Solids	Dextrose concentration	Dextrose/Hb.	Dextrose/Solids	Total dextrose
		gram	°C.	per cent	ratio	per cent	per cent	ratio	per cent	per cent
H4A	Sodium	0.25	+0.8	0.125	.		0.142			
H17	salicylate	0.25	+0.4	0.158	1.51		0.286	2.97		195
H21		0.25	−0.3	0.165	1.42		0.299	2.64		186
55		0.25	−0.1	0.156		0.91	0.243		1.40	154
				0.222*		1.27*	0.218*		1.26*	99*
57		0.25	−0.2	0.177		0.87	0.242		1.21	139
				0.184*		0.91*	0.202*		1.03*	114*
	Average......		+0.4	0.169	1.47	0.99	0.233	2.81	1.23	148
H3	Acetylsa-	0.5	−0.3	0.127			0.182			
H4	licylic	0.5	+0.3	0.136			0.160			
H6	acid	0.5	+0.5	0.134			0.149			
H12		0.25	+0.5	0.138	1.09		0.178	1.30		119
H13		0.5	+0.4	0.136			0.197			127
H22		0.25	−0.4	0.160	1.65		0.184	1.97		129
	Average......		+0.4	0.139	1.37		0.160	1.64		125
H25	Antipyrine	0.2	+0.7	0.109	1.41		0.164	1.88		133
H26		0.2	−0.2	0.157			0.169			110
H27		0.2	+0.4	0.158	1.11		0.141	1.23		110
H29		0.2	−0.8	0.135	1.46		0.166	1.58		113
	Average......		+0.5	0.140	1.33		0.160	1.56		117
H28	Quinine-HCl	0.1	+0.2	0.113	1.12		0.230	2.11		188

* Folin and Wu method.

minations appear in columns five, six and seven; from these it will be seen that our normal dogs with one exception gave an average blood sugar concentration of from 0.11 to 0.18 per cent.

In the sixth column is shown the $\left\{\dfrac{\text{Dextrose}}{\text{Hemoglobin}}\right\}$ ratio and in the seventh column one sees what per cent of the total solids (when these were determined) is represented by dextrose. Conditions at the time of the maximum post-injection dextrose reading are dealt with in columns eight to eleven. Following the maximum dextrose concentrations is seen the corresponding $\left\{\dfrac{\text{Dextrose}}{\text{Hemoglobin}}\right\}$ ratio, or (in the tenth column) the percentage of the dextrose component of the total solids.

After sodium salicylate in five experiments it will be seen that the average maximum dextrose concentration was 0.233 per cent as against a normal average of 0.169 per cent. After acetyl-salicylic acid in six experiments the average maximum dextrose concentration was 0.16 per cent as against a normal average of 0.139 per cent. Similarly four antipyrine experiments gave an increase to 0.156 per cent as against 0.14 per cent, and the quinine result ran as high as 0.23 per cent, or twice the corresponding normal average.

That these changes in the dextrose concentration are due to to a real increase in the total amount of sugar circulating in the blood and not merely to a corresponding loss of fluid in the blood is illustrated in some experiments by the increase in the $\left\{\dfrac{\text{Dextrose}}{\text{Hemoglobin}}\right\}$ ratio, and in others by the $\left\{\dfrac{\text{Dextrose}}{\text{Solids}}\right\}$ ratio. Dividing either of these ratios at the maximum period by the corresponding normal ratio and multiplying the result by 100 one obtains an expression of the percentage change in total blood dextrose. This is illustrated in the last column in which the following averages are seen:

per cent of
normal

Sodium salicylate, maximum total blood dextrose.................. 148
Acetyl-salicylic acid, maximum total blood dextrose.............. 125
Antipyrine, maximum total blood dextrose........................ 117
Quinine, maximum total blood dextrose........................... 188

To indicate the Folin and Wu determinations asterisks are used. While in experiment 55 no increase in blood dextrose

was indicated by this method, this may perhaps be ascribed to the unusually high normal readings.

Fever dogs (summary). The blood dextrose changes in a series of twelve coli fever dogs are illustrated in table 2. From this

TABLE 2

Dextrose in blood of coli fever dogs

DOG NUMBER	DRUG	PER KILO DOSE	BODY TEMPERATURE, MAXIMUM EFFECT	AVERAGE BEFORE DRUG			MAXIMUM AFTER DRUG			
				Dextrose concentration	Dextrose/Hb	Dextrose/Solids	Dextrose concentration	Dextrose/Hb.	Dextrose/Solids	Total dextrose
		gram	*°C.*	*per cent*	*ratio*	*per cent*	*per cent*	*ratio*	*per cent*	*per cent*
H15	Sodium	0.25	−1.0	0.157	1.365		0.373	3.73		272
H16	salicylate	0.25	−0.9	0.126	1.20		0.171	1.78		150
H35		0.25	−0.9	0.129			0.186			
H36		0.5	−0.6	0.137			0.172			
55A		0.25	+0.2 {	0.193		1.02	0.189		1.02	100
				0.146*		0.78*	0.149*		0.81*	104*
60		0.25	−1.4 {				0.208		1..09	
				0.171*		0.78*	0.184*		0.96*	124*
	Average....		−0.8	0.151	1.28	0.86	0.204	2.76	0.97	150
H5	Acetylsa-	0.67	−0.9	0.146			0.242			
H18	licylic	0.25	−1.0	0.173	1.23		0.238	1.85		149
H19	acid	0.25	−1.7	0.125	1.165		0.159	1.63		139
	Average....		−1.2	0.148	1.20		0.213	1.74		144
H33	Antipyrine	0.5	−1.0	0.098	1.06		0.215	2.38		236
57A		0.5	−0.4 {	0.165		0.699	0.186		0.812	116
				0.151*		0.640*	0.150*		0.641*	100*
	Average....		−0.7	0.138	1.06	0.670	0.184	2.38	0.727	151
H32	Quinine-HCl	0.1	−1.4	0.102	1.02		0.202	2.23		219

*Folin and Wu method.

it will be seen that the dextrose concentration of the blood of fever dogs exhibits no detectible difference from that of normal dogs, the averages ranging from 0.10 to 0.19 per cent. The antipyretic drugs under fever conditions increase the dextrose

concentration to an extent similar to that observed in normal dogs.

While the average maximum dextrose concentration after the sodium salicylate injections was only 0.204 per cent it will be seen in six experiments where allowance is made for dilution of the blood that there was a 50 per cent increase in the total dextrose. The total dextrose averages may be summarized as follows:

	per cent of preinjection figure
Sodium salicylate, maximum total blood dextrose	150
Acetyl-salicylic acid, maximum total blood dextrose	144
Antipyrine, maximum total blood dextrose	151
Quinine, maximum total blood dextrose	219

The total blood dextrose on the whole then is increased in the fevered dogs rather more if anything than in the normal dogs. It is significant that in that experiment with sodium salicylate, which failed to give a pronounced fall in temperature but gave instead an increase of 0.2°, determinations by both methods showed no appreciable change in the blood sugar content.

Blood sugar dilution by antipyretics and its significance. From the hemoglobin determinations illustrated in figures 2, 4, and 6, as well as the total solids determinations in figures 7 and 8, it becomes evident that antipyretic drugs dilute the blood simultaneously with the temperature reduction. The experiments on the normal dogs as well as the observations of others, for example Hanzlik's (14) hemoglobin determinations after salicylates, all combine to show that no significant effect is exerted under normal conditions (in figures 1, 3 and 5 a slight blood concentration is indicated, associated with some temperature increase).

It is known that dilution of the blood favors reduction in temperature by affording a greater volume for radiation from the body periphery; if also the swelling pressure of the blood decreases, this promotes sweating and, in cases of increased ventilation, evaporation from the lungs.

In figures 9 and 10 are illustrated further the effects of sodium salicylate injections in dogs two and one-half to three hours
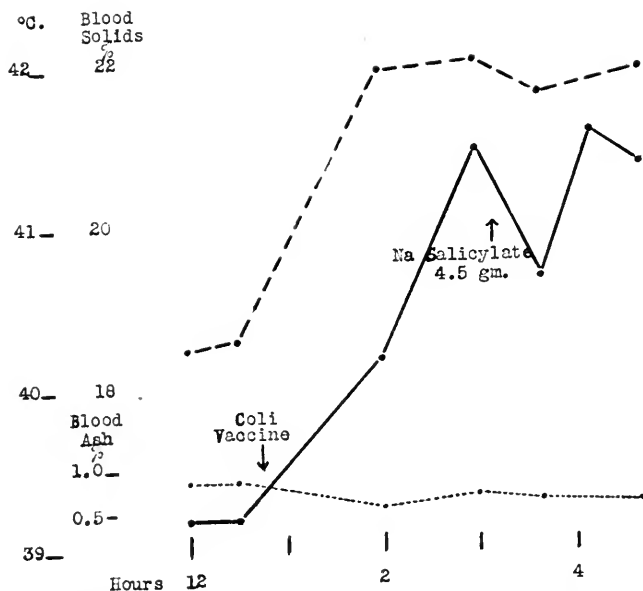
FIG. 9. EXPERIMENT 24. EFFECT OF COLI VACCINE AND OF SODIUM SALICYLATE

Rectal temperature: continuous line. Blood solids per cent: heavy broken line. Blood ash per cent: light broken line.
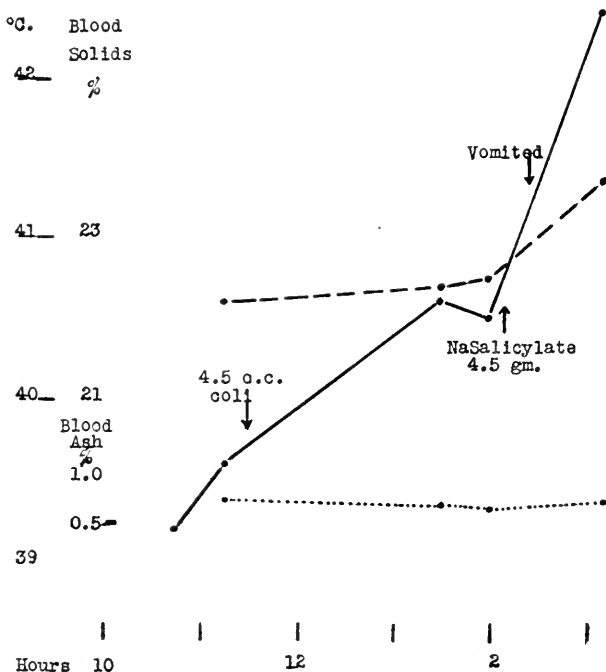
FIG. 10. EXPERIMENT 25. Effect of COLI VACCINE AND OF SODIUM SALICYLATE

Rectal temperature continuous line. Blood solids per cent: heavy broken line. Blood ash per cent: light broken line. Note effect of vomiting after salicylate.

178

after coli injections, that is to say, at the height of coli fever. In both cases is seen the increase in blood solids which Barbour and Howard have shown to be a typical result of coli injection. In figure 9 blood dilution is seen to result as usual from the salicylate injection. In the experiment illustrated in figure 10, however, the salicylate induced vomiting. This was associated with further loss of fluid from the circulation, as the curve of blood solids shows, and with another increase in the body temperature.

Blood salts. In both of the last mentioned experiments the blood was ashed to determine whether or not changes in blood salts run parallel to the fluid changes under the conditions. Some diminution in the total blood ash was seen as a result of the coli injections; thus the inorganic constituents appear to leave the blood along with the water. However, after the sodium salicylate injections, slight changes opposite in direction from the water shifting were noted.

Effects of antipyretics upon the blood concentration in fever patients. In connection with metabolism studies on antipyretic drugs in the New Haven Hospital a number of hemoglobin determinations were made before and after administration of acetyl-salicylic acid to fever patients. The conditions were nearly ideal, as the individuals were at the time serving as subjects for metabolism determinations; they had been fasted, except for water, since the previous evening and were kept nearly motionless throughout the entire period of observation.

From the results, which are presented in table 3, it is seen that doses of acetyl-salicylic acid sufficing to reduce fever temperature by from 0.2 to 1.2°C. diminished the hemoglobin content of the blood by from 6 to 13 per cent. Furthermore the degree of diminution appears roughly proportional to the fall in temperature. As this rapid change is undoubtedly due to dilution we have confirmed in man the contention that antipyretic drug action is brought about by increasing the water content of the blood.

TABLE 3

Effect of acetyl-salicylic acid upon the hemoglobin during antipyresis in fever patients

SUBJECT	DIAGNOSIS	DATE	TIME	ACETYL-SALICYLIC ACID	TEMPERATURE	HEMOGLOBIN	
						Standard	Change
				gram	°C.	per cent	per cent
J. M.	Tuberculosis and ascites	11–29–19	11:14		38.20	103	
			11:20	0.75			
			12:22		37.98	96	−7
		12–6–19	9:45		38.0		
			10:40			100	
			10:45	1.0			
			12:40		37.32	87	−13
C. H.	Tuberculosis (?)	12–3–19	10:00		38.8		
			11:05			83	
			11:20	0.75			
			11:30			78 } 74	
			12:45			73	
			1:00			71	
			1:20		37.6		−11
F. R.	Paratyphoid	12–8–19	10:30		37.68	111	
			10:59	1.0			
			12:15			100	
			12:35		36.88		−10
L. W.	Acute tonsillitis	12–13–19	12:44		37.8		
			1:00			143* } 145	
			1:15	0.75		147	
			1:30				
			2:20			133 } 136	
			3:00		37.5	139	−6

* High readings due to deterioration of standard.

This does not detract from the relative value of this and the other three readings.

DISCUSSION

Effects of other substances which dilute the blood. Dextrose given intravenously to coli fever dogs produces temporarily a sharp fall in body temperature associated with a similar decline in the curve of blood solids. Much greater dilution of the blood occurs than when a similar injection is made in normal

dogs, which correspondingly exhibit no decrease in the body temperature (Barbour and Howard (5)). Similarly such intravenous acacia injections as fail to reduce the temperature of normal rabbits and even increase that of normal dogs, exert a profound antipyretic effect in both peptone and heat puncture fever rabbits and in coli fever dogs (Barbour and Baretz (19)). In the latter case also, the antipyretic effect in two experiments was shown to be associated with dilution of the blood. This, however, could safely be assumed from what is known of the effects of acacia upon the blood volume (cf. Smith and Mendel (20)).

How does the blood become diluted? It is not possible at this time to state definitely exactly what factors are responsible for the blood dilution by antipyretics in fever. The analogy with the dextrose effects, coupled with the above demonstration of the sugar mobilizing capacity of antipyretic drugs, has led us to suggest that the increase in blood dextrose is one of the chief factors. There certainly must be, as Woodyatt (6) and others have often suggested, a greater percentage of available water in the tissues in fever than in health, or in other words, febrile conditions must be associated with a low swelling pressure of the tissues. This accounts for the source of the water. Whether enough sugar is poured into the blood as a result of the antipyretic drug action to attract any of this water into the circulation again, cannot at present be stated.

That the combustion of sugar is not constantly altered by antipyretic drugs is the final deduction from a series of experiments upon man soon to appear from this laboratory. The possibility of the inhibition of glycolytic enzymes by these drugs deserves investigation.

Explanations of the blood dilution not dependent upon the dextrose content should also be entertained, for example increased permeability of the capillaries; this however always leaves open the question as to why the increased permeability favors a flow in one direction more than in the other. A reduction in the supposedly increased hydration capacities of the tissue colloids would also account for the loss of water to the blood. But the dextrose mobilization will, until other mecha-

nisms have been demonstrated, afford an explanation of anti-pyretic action that is at least plausible.

It is perhaps unnecessary to add that the low grade of increase in the dextrose concentration is possibly more favorable to hemo-dilution than a greater blood sugar increase would be; for a high sugar content of the blood, especially if continued for any appre-ciable length of time, tends to a marked diuretic effect, leading ultimately to Woodyatt's sugar dehydration fever.

Theory of the mechanism of fever reduction by drugs. The prob-able mechanism of antipyretic drug action may be summarized as follows: All antipyretics act by increasing the heat elim-ination; reduction in heat production is incidental (21). Anti-pyretics increase the blood sugar concentration. This in fever causes a plethora, extra water being available in the tissues. Plethora promotes the dissipation of the heat by radiation and surface evaporation, for the peripheral blood flow becomes aug-mented. In health no plethora occurs—consequently there is no antipyretic effect.

CONCLUSIONS

1. Sodium salicylate, acetyl-salicylic acid, antipyrine and qui-nine all increase the blood sugar concentration in both normal and fever dogs. After salicylates the total blood dextrose increase, as determined by the Benedict method, amounts to from 25 to 50 per cent. The increase appears smaller when estimated by the method of Folin and Wu. Antipyrine apparently gives a less marked effect than do salicylates, while quinine exhibits a greater.

2. In coli fever dogs as well as in human febrile cases these drugs produce a notable dilution of the abnormally concen-trated blood. This dilution accounts for the decrease in body temperature.

3. In normal dogs antipyretic drugs often increase the body temperature slightly, in which case they may diminish the fluid content of the blood.

4. According to the theory of antipyretic action evolved from our work the mobilization of dextrose is one of the chief factors responsible for the blood dilution by antipyretic drugs in fever.

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THE ACTIVITY OF THE ISOLATED UTERUS

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The following experiments were undertaken to analyze the automatic activity of the isolated uterus, and to determine to what extent this activity is modified by severing the nervous connection of the uterus.

Cushny (1) examined the movements of the uterus of the cat and rabbit in situ, and Kehrer (2) analyzed those of the isolated organ of the cat, rabbit and dog in the virgin and pregnant animal. They showed that in the virgin cat and rabbit the movements were of two kinds: 1, pendulum movements and 2, tonic changes.

Kehrer found that small portions of the isolated horn showed regular pendulum movements, which were shown by very small isolated pieces of the horns and were obtained when either the longitudinal or circular muscle was recorded. The cervix of the uterus when isolated from the horns showed less frequent pendulum movements. In the pregnant cat the pendulum movements were stronger, more irregular and less frequent. He found that the same changes of movement during pregnancy occurred both in those parts of the horn that contained a fetus, and in the remainder of the horn; moreover when one horn contained embryos and the other horn was empty, the same kind of contractions were shown by both sides.

Kurdinowski (3) and Kehrer both describe peristaltic movements passing downwards from the distal portion of the horn to the cervix of the uterus, but Cushny did not find any regular peristaltic movements, sometimes a part of the horn contracting and sometimes the whole.

With regard to the nervous control of the uterus Langley and Anderson (4) showed that both motor and inhibitory fibers pass from the sympathetic chain to the hypogastric nerve, and concluded that the cell stations were scattered irregularly in the inferior mesenteric ganglion and along the nerves. They state that nicotine paralyzes the effects of stimulation of the hypogastric nerve, while Cushny showed that nicotine does not inhibit the normal movements of the uterus. These can be modified by stimulation of the central ends of most sensory nerves, and Barbour (5) found them altered by cooling or heating the cerebral cortex in the pregnant rabbit. Kurdinowski observed birth occurring in the isolated uterus of the rabbit; this shows that the most complicated series of movements that the uterus performs can be executed without the interference of the central nervous system.

Kehrer showed that the two horns of the cat performed pendulum movements independently and that the rate of pendulum movement was more rapid at the ovarian than at the uterine end of the horns.

EXPERIMENTS

Observations on the excised uterus showed the different parts of a complete horn in different states of activity, part being relaxed and part contracted at the same moment. The movements recorded by an entire horn represent therefore the sum of the activities of different parts of the horn, some parts of which are contracting and others relaxing.

I therefore used small portions of the uterine horn 0.5 to 3 cm. and recorded the contractions of both the longitudinal and circular fibers with double levers. The isolated pieces of the organ were suspended in oxygenated Ringer's fluid in the usual manner. Temperature of bath 37°C. Composition of Ringer NaCl 0.9; $CaCl_2$ 0.024; KCl 0.042; $NaHCO_3$ 0.016; PH 8.0. The volume of the bath was 150 cc.

Experiments were made chiefly on rabbits, and a few on guinea-pigs and rats. Most experiments were made upon virgin animals, and a few upon pregnant and multiparous animals.

I. Nature of pendulum movements

Pieces of uterine horn of virgin rabbits were suspended in the manner shown in figure 1 to determine if the different portions of the horn showed the same rate of rhythm figure 2 shows the
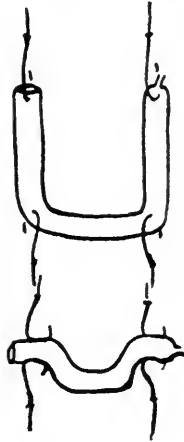
FIG. 1
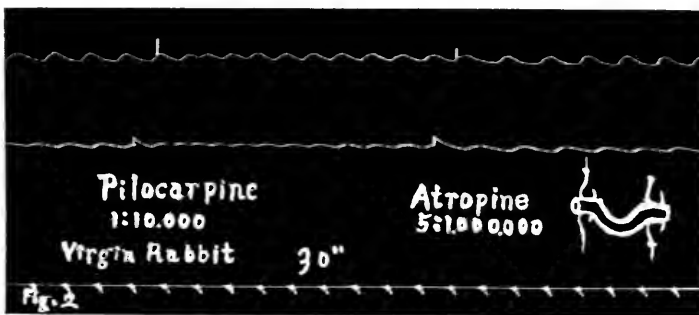
FIG. 2. RHYTHMIC CONTRACTIONS OF CIRCULAR MUSCLE COAT OF VIRGIN
RABBIT UTERUS

Uterus not divided. Upper tracing: Vaginal end, rate one contraction in thirty-two seconds.   Lower tracing: Ovarian end, rate one contraction in twenty-seven seconds.

movements obtained from the circular mcleus in a virgin rabbit and figure 3 from the longitudinal muscle in a multiparous non-pregnant rabbit. Figure 2 shows that the different portions of

the uterus contract at different rates, the ovarian end contracting more rapidly, therefore the pendulum movements cannot pass down the horns as peristaltic waves. Figure 3 suggests that general tonic changes affect the whole length of the uterus, a point observed by Cushny. In other experiments the horn was cut between the two points of suspension and no alteration was
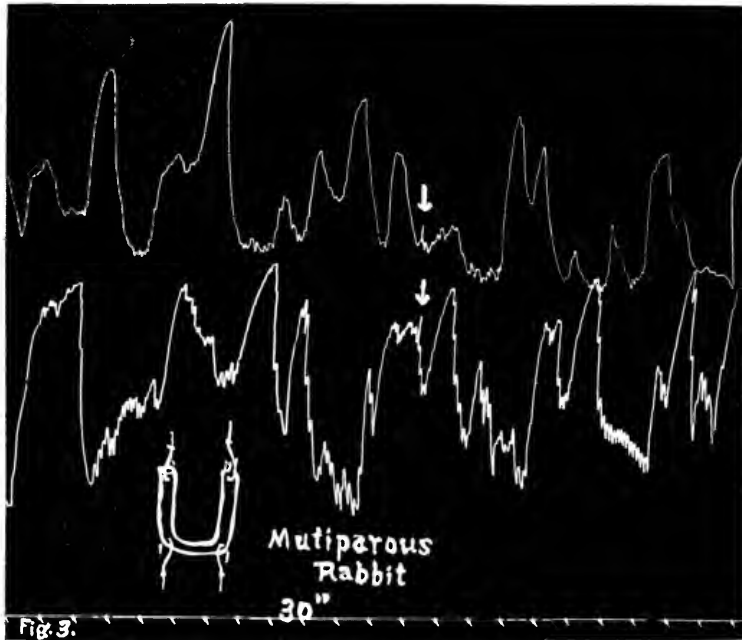
FIG. 3. UTERUS OF NON-PREGNANT MULTIPAROUS RABBIT

Uterus not divided. Longitudinal muscle recorded. Upper tracing: Ovarian end. Lower tracing: Vaginal end.

observed in the activity of the two portions. Moreover pieces of longitudinal and circular muscle recorded simultaneously from the same region of the horn did not contract at the same rate. Finally the rate of pendulum movements is modified by the length of strip taken. In one case a uterine horn in a multiprous rabbit was split and 4.5 cm. of one-half taken and 1.8 cm. from the middle of the other half, and the shorter piece contracted about

twice as rapidly as the longer piece (fig. 4). Figure 5 shows records taken from two points in the horn of the multiparous rabbit, in this case both portions contracted exactly simultaneously, and therefore the contractions were not of a peristaltic

FIG. 4. MULTIPAROUS RABBIT. HOUR OF THE UTERUS SPLIT

Upper tracing: Longitudinal strip 4.5 cm. Lower tracing: Longitudinal trip 1.8 c.m.

FIG. 5. MULTIPAROUS RABBIT. WHOLE UTERUS HORN AS IN FIGURE 1

Upper tracing: Longitudinal muscle, ovarian end. Lower tracing: Longitudinal muscle, vaginal end.

nature. Apparently therefore the uterine periodic contractions are not of a peristaltic nature, and in the virgin rabbit each portion of the muscle performs small pendulum movements independently. In the multiparous and pregnant rabbit the contractions are larger, less frequent and more irregular but frequently the whole horn passes into simultaneous contraction.

II. Activity of virgin and pregnant uteri

The chief differences observed between the virgin and pregnant
uteri of rabbits were that the contractions of the virgin uteri
were more frequent, more regular and less powerful.   An example
of the contractions of a small piece of pregnant rabbits' uterus
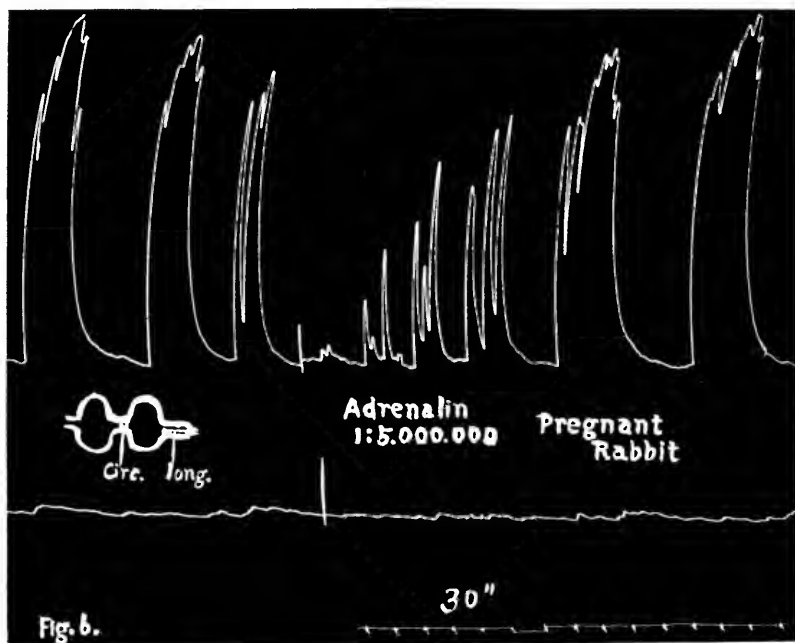
FIG. 6. CONTRACTIONS OF LONGITUDINAL AND CIRCULAR MUSCLE OF PREGNANT
RABBIT

is shown in figure 6.   It will be seen that the longitudinal muscle
contracts vigorously and that the circular muscle contracts
scarcely at all.   The longitudinal fibers show a double rhythm,
large contractions occurring every two to three minutes and
lasting about a minute, and superimposed small contractions
occurring every fifteen seconds.

III. *Response of virgin rabbit's uterus to drugs*

The response of the rabbit's uterus to the usual pharmacological agents is sufficiently well established to render it unnecessary to give detailed references. Kehrer pointed out that the uterus could respond in two ways to a drug either by changes in the rate and force of the pendulum movements, or by changes in tonus, or changes in both might occur. By using small pieces of uterus it was possible to analyze exactly the changes occurring when drugs were given.
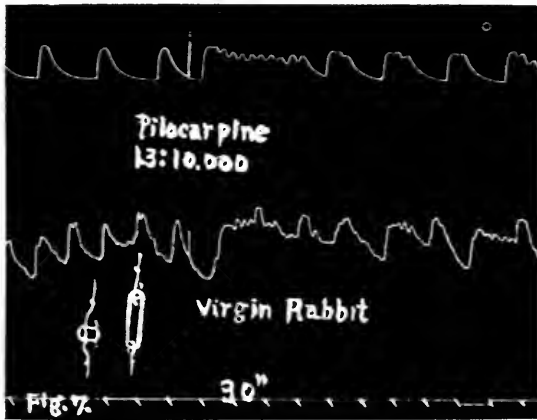
FIG. 7. VIRGIN RABBIT UTERUS SUSPENDED AS IN DIAGRAM

Upper tracing: Circular muscle.   Lower tracing: Longitudinal muscle

I found that a considerable individual variation in the response of the uterus to drugs occurred. Pilocarpine sometimes caused an increase in rate alone (fig. 2) and sometimes caused an increase in tonus (fig. 7). Atropine abolished the action of pilocarpine (fig. 2), but when given without any previous administration of pilocarpine produced very little effect. The action of adrenalin was complicated. In the longitudinal muscle it caused an increase in tonus and in rate (fig. 8), whereas in circular muscle it caused usually an increase in the rate of contraction (fig. 9), but after the administration of atropine, adrenalin always produced a temporary inhibition both of the
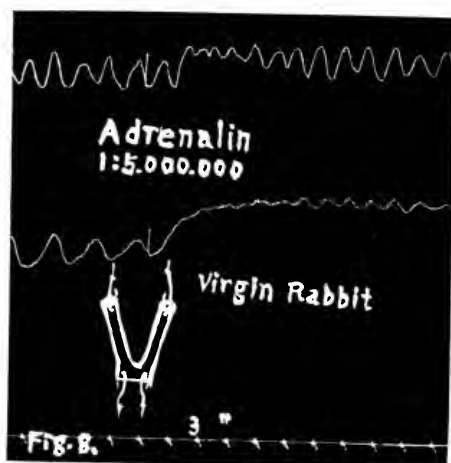
FIG. 8. VIRGIN RABBIT. NERVES TO RIGHT HORN DIVIDED EIGHTY-TWO DAYS
PREVIOUSLY

Uterus and horns excised as in diagram. Upper tracing: Right operated
horn, longitudinal muscle. Lower tracing: Left normal horn, longitudinal
muscle.
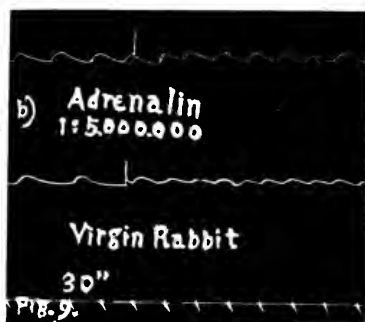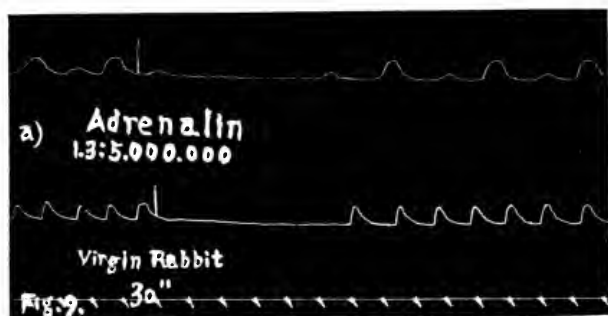
FIG. 9. VIRGIN RABBIT UTERUS

a. Action of adrenalin after atropine. Virgin rabbit, nerves of right
horn divided sixty-four days previously. Upper tracing: Normal left horn,
circular muscle. Lower tracing: Operated right horn, circular muscle.

b. Action of adrenalin. Virgin rabbit nerves of right horn divided sixty-five
days previously. Upper tracing: Normal left horn, circular muscle. Lower
tracing: Operated right horn, circular muscle.

circular and the longitudinal muscle (fig. 9). After ergotoxine adrenalin always produced inhibition of both the circular and longitudinal muscle. The action of atropine therefore appears to
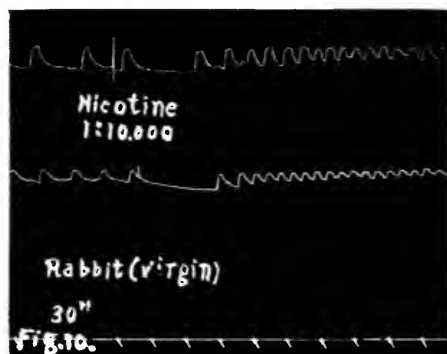
FIG. 10. VIRGIN RABBIT. OPERATED FIFTY-SIX DAYS PREVIOUSLY

Nerves to right horn cut. Upper tracing: Circular muscle, normal left horn. Lower tracing: Circular muscle, operated right horn.
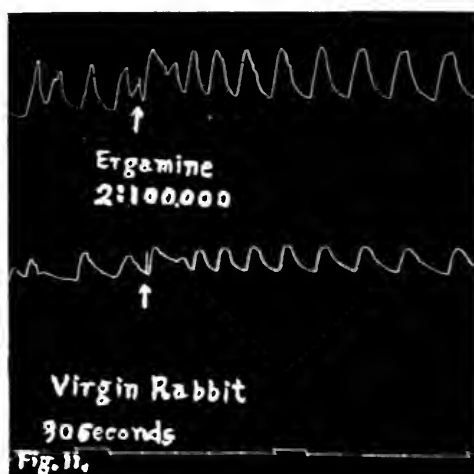
FIG. 11. VIRGIN RABBIT'S UTERUS

Upper tracing: Longitudinal strip. Lower tracing: Circular strip

resemble the final action of ergotoxine in the uterus, in that it paralyzes the motor endings of the sympathetic. Nicotine often caused a temporary inhibition of the uterus followed by an

increase in the rate of contraction (fig. 10). Ergamine caused a slight increase in the rate of contraction but no marked tonic changes in either the circular or longitudinal coat (fig. 11). Pituitary extract often producing a well marked inhibitory action
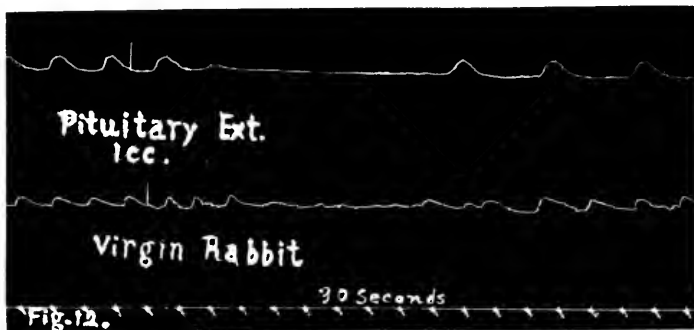
FIG. 12. VIRGIN RABBIT. NERVES OF RIGHT HORN CUT SIXTY-FOUR DAYS PREVIOUSLY

Upper tracing: Normal horn, circular muscle. Lower tracing: Operated horn, circular muscle.
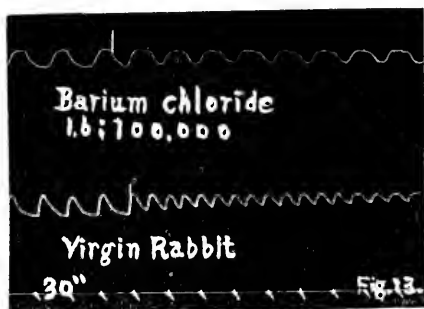
FIG. 13. VIRGIN RABBIT. RIGHT HORN CUT SIXTY-FOUR DAYS PREVIOUSLY

Upper tracing: Normal left horn, circular muscle. Lower tracing: Operated right horn, circular muscle.

(fig. 12) although usually an increase of tonus was observed. Barium chloride always caused a definite increase of tonus and increase in frequency of movements (fig. 13).

These experiments show that the virgin rabbit's uterus usually responds to mild excitation by an increase in frequency of move-

ments rather than by extensive tonic changes, and that the response appears to be frequently of a mixed type, initial inhibition followed by stimulation; moreover different animals show different response to the same drugs, in some cases the inhibitory action being much more pronounced than in others.

IV. Response of uterus of pregnant or multiparous rabbits to drugs

The uterus of the pregnant or multiparous rabbit usually reacts to stimulant drugs by extensive tonic changes. In many cases the pendulum movements are so irregular that changes in rate
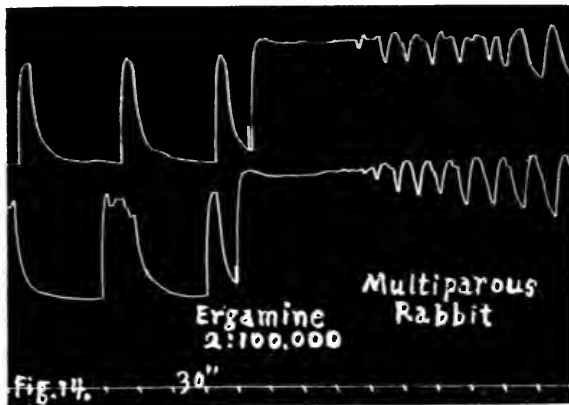
FIG. 14. MULTIPAROUS RABBIT. NERVES OF RIGHT HORN CUT TWENTY-TWO
DAYS PREVIOUSLY

Upper tracing: Left normal horn, longitudinal muscle. Lower tracing: Right operated horn, longitudinal muscle.

cannot be estimated, but drugs that stimulate the uterus usually cause an increase in the rate of pendulum movements. Figure 14 shows the response to ergamine of the uterus of a multiparous rabbit, this shows a marked contrast to the response of the virgin rabbit's uterus (fig. 11). Similarly pituitary extract causes a marked tonic contraction in pregnant or multiparous animals (fig. 15).
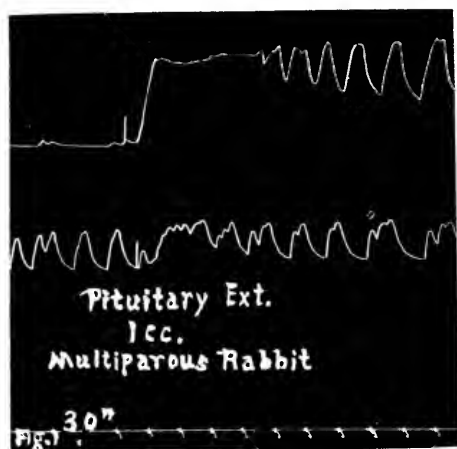
FIG. 15. MULTIPAROUS RABBIT. NERVES TO RIGHT HORN CUT TWENTY-TWO
DAYS PREVIOUSLY

Upper tracing: Normal left horn, longitudinal muscle. Lower tracing:
Operated right horn, longitudinal muscle
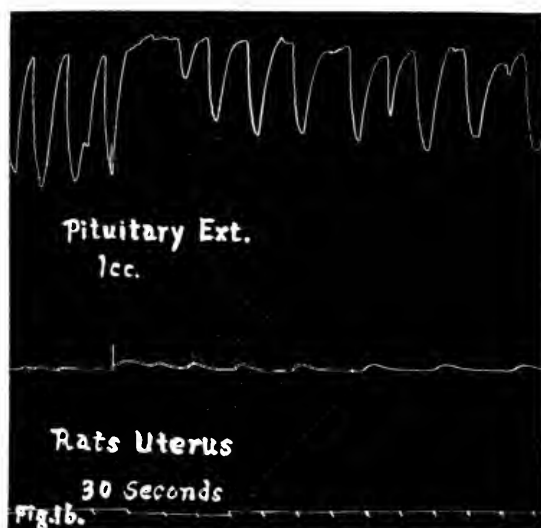
FIG. 16. RAT'S UTERUS

Upper tracing: Longitudinal muscle.   Lower tracing: Circular muscle

V. *Uterus of rat*

Small pieces of the rat's uterus show pendulum movements closely resembling those of the rabbit (fig. 16). In the rat the inhibitory fibers are stronger than in the rabbit, and as shown by Gunn (7), ergamine and adrenalin cause relaxation of the uterus, although pituitary extract causes contraction (fig. 16).

VI. *Uterus of guinea-pig*

Only a few experiments were made with guinea-pigs, but in the young virgin feeble pendulum movements were observed, whereas in the pregnant and multiparous animal occasional powerful contractions occurred as in the pregnant and multiparous rabbit. As a whole the virgin guinea-pig's uterus showed a much greater tendency to extensive tonic changes than did the virgin rabbit's uterus.

VII. *Relation of uterine movements of nervous supply*

Owing to the irregular course taken by the uterine nerves it is not possible to be certain of destroying all nerves without at the same time destroying the blood supply to the uterus. In thirteen rabbits I severed the nervous supply of the uterus as far as possible by cutting the broad ligament as is shown in figure 17. After periods varying from twenty-two to eighty-two days to allow full time for the nerves to degenerate the uteri were excised, records taken of their response to drugs, the response of the operated side being compared with that of the normal side. Figures 8, 9, 10, 12, 13, 14, and 15 illustrate the response of the two sides to various drugs.

In nearly every case the operated side showed more frequent and stronger automatic movements than the normal side. This difference in movement between the operated and normal sides indicates that the nervous connection of the operated side had been destroyed. In all cases the response to drugs of the normal and operated side was of the same nature. For instance in figure 9 in one rabbit both horns responded to adrenalin by increased rate of contraction, and in the other case after atropine both

sides responded to adrenalin by inhibition.   In most cases the
effect produced by drugs was more marked on the operated than
on the normal side.   This agrees with the observations of Elliott
(8) and others who have shown that section of nerves renders
the sympathetic nerve endings more susceptible to stimulation.
Figure 13 however shows that barium chloride which acts on
muscle and not on nerve endings also causes a greater response
on the operated than in the normal horn.   The difference in
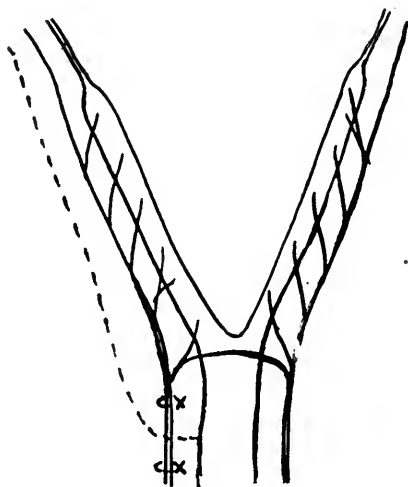
Fig. 17

response to drugs therefore cannot be attributed with certainty
to any sensitivity of the nerve endings, but must be due to some
alteration in the tonus of the muscle.

VIII.   Presence of ganglion cells in the uterus

Langley and Anderson concluded that the cell stations of the
sympathetic nerve are scattered irregularly in the inferior mesen-
teric ganglion and along the hypogastric nerves.   Numerous
workers have looked for ganglion cells in and around the uterus.
Ganglion cells have been found in the subperitoneal tissue and
in the outer muscle layer of the dog's uterus (la Torre (9)), in the
uterus of a two months' baby (Keiffer (10)) and in the paramet-

rium of the human uterus (Kilian and Stöhr (11)). I examined rabbits' uteri by means of the vital staining method with methylene blue, and also by Bielschowski's method but with negative results. If ganglion cells exist in the rabbit uterus they must be few in number, it is certain that no nerve plexus containing numerous ganglion cells occurs in the uterus of the rabbit.

SUMMARY

1. The pendulum movements of the excised uterine horn of the virgin rabbit are not transmitted along the horn in the manner of a peristaltic wave but each part of the uterus contracts independently.

2. In the excised uterus of the pregnant or multiparous rabbit frequently the whole horn contracts simultaneously, but often different portions show independent activity.

3. The uteri of different virgin rabbits show considerable variations in their reaction to adrenalin.

4. After atropine adrenalin produces a well marked inhibition in the virgin rabbit's uterus.

5. Degenerative section of the nerves supplying the uterus slightly increases the automatic activity of the uterus and also slightly increases the response of the uterus to all drugs which cause increased contraction, whether these act on nerve endings like adrenalin, or on muscle like barium.

6. I have been unable to discover any nerve ganglion cells in the rabbit's uterus.

7. All parts of the rabbit's uterus, virgin, pregnant, or multiparous show automatic rhythmic contractions however small a piece is taken.

8. The circular muscle responds to drugs in every case in the same manner as the longitudinal muscle.

9. All the above facts suggest that the automatic activity of the uterus is of myogenic and not neurogenic origin.

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NOTES ON THE TOXIC EFFECTS OF CHLORINE ANTISEPTICS IN DOGS

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The following report offers quantitative data and other observations relating to the toxicity of two of the chlorine antiseptics, Dakin's solution and chloramine-T,[1] especially when injected into the peritoneum.

The dangers of application of chlorine antiseptics to serous membranes have been brought out by Grey (1), who found an inflammatory reaction proportional to the chlorine content of the antiseptic introduced into the peritoneal cavity. Hartwell and Butler (2), however, demonstrated the value of Dakin's solution in surgical empyemas when contact of the solution with uninjured pleural surfaces can be avoided.

Particular interest attaches to the toxicity of the most stable of the water-soluble chlorine antiseptics, namely, chloramine-T. Previous reports on this point appear conflicting. From the figures of Taylor and Austin (3), who gave chloramine-T intraperitoneally in mice, it would appear to be just as toxic as corrosive sublimate and twelve times as much so as Dakin's solution. Taylor and Austin's figures are:

	Minimal lethal dose for mice intraperitoneally; mgm. per kilo
Mercuric chlorid	15 (5–25)
Chloramine-T (NaOCl equivalent)	17 (8–26)
Dakin's solution (NaOCl equivalent)	180 (120–240)

Given by other routes chloramine-T appears comparatively harmless. For example, Fantus and Smith (4) found the sub-

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[1] Chloramine-T (Calco) was used throughout the work.

cutaneous minimal lethal dose for mice and guinea-pigs respectively 300 and 900 mgm. per kilo. In terms of the NaOCl equivalent the figures would be approximately 75 and 225 mgm. Intravenously, 25 mgm. per kilo killed one rabbit while two rabbits given twice this dose survived.

By mouth, Fantus and Smith gave 400 mgm. per kilo (10 cc. of a 4 per cent solution) to a dog. Profuse and repeated vomiting resulted, lasting for thirty minutes. Twenty cubic centimeters of

TABLE 1

Toxicity of chloramine-T injected intraperitoneally in dogs

NUM-BER	DOG	CHLORAMINE–T (2%)			FATE	PERITONEAL FLUID
		Cubic centimeters of solution	Milligrams per kilo	NaOCl equivalent milligrams per kilo		
	kilos					*cc.*
1	18.0	29	32^1	9^1	Recovered	400 (16th day)
		58	64^2	17^2	Recovered	
		116	128^3	34^3	Recovered	
		310	380^4	100^4	Recovered	
7	18.0	58	64	17	Recovered	40 (15th day)
16	10.0	48	95	25	Recovered	
20	6.0	29	95	25	Recovered	
21	13.0	62	95	25	Recovered	
15	10.7	69	128	34	Died within 36 hours	200+
5	14.0	90	128	34	Died within 18 hours	250
2	10.8	205	380	100	Died within 4.5 hours	450

[1,2,3,4] First, second, third and fourth injection in same dog at intervals of two days.

0.4 per cent produced vomiting only after seven hours. The first instance shows that chloramine-T probably produces no serious injury when given by stomach in large quantities and high concentrations. It is said to become decomposed in the gastric juice and the general disposition is to regard it as non-toxic, the antiseptic being found useful for example as a mouth wash.[2]

[2] New and Non-Official Remedies, 1921, p. 144.

Normal dogs were used in the present work; the intraperitoneal injections were made slowly by gravity assisted by pressure from a hand bulb.

The toxicity of chloramine-T given in this manner is illustrated in table 1. The first two columns relate to the number and weight of the animal; the next three to the antiseptic which was given in 2 per cent solution, indicating respectively the total amount

TABLE 2

Toxicity of Dakin's solution injected intraperitoneally in dogs

DOG		DAKIN'S SOLUTION				FATE	PERITO-NEAL FLUID
Num-ber	Kilos	Amount	"Available chlorine"		NaOCl equiva-lent		
		cc.	per cent	mgm. per kilo	mgm. per kilo		cc.
24	6.1	48	0.454	36	37.5	Recovered	
25	6.0	48	0.454	36	37.5	Recovered	
18	9.0	120	0.376	50	52.5	Recovered	
19	8.0	106	0.376	50	52 5	Died within 36 hours	355
23	8.4	92	0.454	50	52.5	Recovered	
17	11.0	195	0.376	67	70.0	Recovered	
12	9.7	152	0.427	67	70.0	Died within 50 hours	180
6	10.5	210	0.400*	100*	104.5*	Died within 24 hours	200
11	10.0	234	0.427	100	104.5	Killed 28th day†	35
22	7.2	158	0.454	100	104.5	Died in 24 hours	105

* Approximate.

† Had refused food for ten days. Great emaciation. Chronic peritonitis.

of solution given, the number of milligrams per kilo and the approximate sodium hypochlorite equivalent. In the sixth column is indicated the fate of the animal and in the last column is shown the amount of peritoneal fluid present at death or after recovery. As regards the toxicity, it is evident that the minimal lethal dose lies between 95 and 128 mgm. per kilo or between the hypochlorite equivalents of 25 and 34 mgm.

Dakin's solution injected intraperitoneally gave less definite results as regards toxicity (table 2). In this table, the fourth and fifth columns relate to the content of the solution in "available chlorine," giving respectively the percentage as determined by

the method of Dakin, and the calculated milligrams per kilo. In the sixth column appears the sodium hypochlorite equivalent also in terms of milligrams per kilo.

The minimal surely lethal dose appears to lie between 67 and 100 mgm. available chlorine per kilo, although one animal (No. 6) receiving the latter amount was able to survive for twenty-eight days and might have lived two or three days longer. On the other hand one injection of 50 mgm. per kilo was fatal. The lowest dose which can surely be survived therefore probably lies between 37.5 and 52.5 mgm. per kilo sodium hypochlorite, which would

TABLE 3

Toxicity of mercuric chloride injected intraperitoneally in dogs

DOG		MERCURIC CHLORIDE (0.1 PER CENT)		FATE	PERITONEAL FLUID
Number	Kilos	Cubic centimeters of solution	Milligrams per kilo		
					cc.
10	10.0	40	4	Recovered	
13	9.7	39	4	Recovered	
14	9.0	72	8	Died within 36 hours	160
4	16.0	272	17	Died within 24 hours	
3	13.6	462	34	Died within 3.5 hours	250

make Dakin's solution certainly no more than twice as safe (given intraperitoneally) as chloramine-T. These results, both differing considerably from those of Taylor and Austin in mice, it was thought of interest to compare them, as did the last mentioned authors, with the results of intraperitoneal injections of mercuric chlorid. This is done in the five experiments summarized in table 3. The concentration selected was 1:1000. The table shows that the minimal lethal dose lies between 4 and 8 mgm. per kilo.

Summarizing the toxicity of the three above-discussed antiseptics, given intraperitoneally to dogs:

Minimal lethal dose; mgm. per kilo

Chloramine-T (NaOCl equivalent)...................... 30 (25–34)
Dakin's solution (NaOCl equivalent)................... 45 (37.5–52.5)
Mercuric chlorid...................................... 6 (4–8)

According to these figures corrosive sublimate is five times as toxic as chloramine-T, which in turn is but one and one-half times as toxic as Dakin's solution.

OTHER OBSERVATIONS

Peritonitis. Varying stages of acute or chronic peritonitis were found in these animals, according to the interval between the injection and its fatal termination or the killing of a recovered dog. These were, in the case of all three antiseptics, of the type of aseptic inflammation ordinarily to be expected from a corrosive substance—serous or sero-hemorrhagic, then fibrinous, then fibrous elements predominating.

From the amount of peritoneal fluid obtained it would appear that chloramine-T was the only one of the substances provoking a marked net loss of fluid from the circulation to the peritoneal cavity. In all three fatal cases at least twice as much fluid was found as had been injected (table 1). In the case of Dakin's solution the three animals succumbing to the larger doses within one or two days showed no special change in the amount of peritoneal fluid. In the one which died with the smallest dose, however, the amount of peritoneal fluid was almost tripled. In the case of fatalities from corrosive sublimate, in the two instances measured the peritoneal fluid was found to be approximately doubled in one case and halved in the other.

Clinical symptoms. All of the animals receiving fatal injections of the antiseptics exhibited from the very beginning marked muscular depression and lassitude with complete loss of appetite and weakness of pulse.

A reduction in body temperature was constantly found. In the case of chloramine-T the reduction in temperature before the animal became moribund amounted to 2.4 to 3.5°C. Dakin's solution provoked temperature reductions of 1 to 2°C. in cases of recovery and 1 to 4°C. in fatal cases. Mercuric chlorid in the sublethal dose provoked a temperature fall of 1°C., while in one of the fatal cases the fall was 6°C. before the dog was moribund.

Effects of giving chloramine-T by mouth. The comparative harmlessness of chloramine-T given by mouth to dogs was illustrated by two experiments. Both were performed on 10 kgm.

dogs, one being given 128 cc. of 1 per cent chloramine-T, the other 64 cc. of 2 per cent chloramine-T. Both dogs vomited for a few minutes and then recovered without further event.

CONCLUSIONS

1. Given intraperitoneally in dogs, chloramine-T appears to possess one and one-half times the toxicity of Dakin's solution and but one-fifth the toxicity of corrosive sublimate.

2. All three of these antiseptics produce acute or chronic peritonitis, the severity depending upon the amount injected.

3. Fatal intraperitoneal injections of chloramine-T appear not only to fail of absorption but to attract in addition at least an equal amount of fluid from the circulation.

4. All of the above-mentioned antiseptics produce circulatory and muscular collapse with reduction in body temperature.

5. Large amounts of 2 per cent chloramine-T by mouth can be tolerated by dogs without other significant effect than vomiting.

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THE REACTION TO EPINEPHRIN ADMINISTERED BY RECTUM

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In connection with other investigations, it was desired to find some way of administering epinephrin so that it would be rather slowly absorbed but still enter the circulation rapidly enough to produce significant effects. The oral route has been found unsatisfactory, the drug apparently being destroyed before reaching the blood-stream. Hypodermatic injections meet some requirements but for long-continued researches are not in all ways satisfactory. Absorption is rather too slow and the danger of local necrosis is not negligible.

In view of the relatively profuse circulation of the lower rectum, it seemed probable that from this site the drug might be absorbed fairly rapidly and directly into the veins emptying into the vena cava. In short, it appeared that intrarectal injections might in a measure simulate normal discharge from the suprarenal glands.

The pharmacodynamic reactions to epinephrin administered by rectum have apparently been but little investigated. Binet found that when so administered the drug was very toxic; hence it appears that it must have been absorbed fairly freely. Lesné has more recently reported some observations on human subjects. When administered to children in 2 mgm. doses, or to adults in 3 or 4 mgm. doses, it evoked a vasomotor reaction comparable to that of intravenous infusions in experimental animals. In some cases there was a slow rise of pressure reaching its maximum in fifteen to twenty minutes, and, in some cases, a slight fall was observed. Recently, Muirhead has reported some observations on the therapeutic use of epinephrin in Addison's disease. Two cubic centimeters of 1:1,000 solution administered by rectum

resulted in a rise of pressure from 98 to 110 mm. and this persisted about an hour before the original level was regained. Since the observations herein recorded were made, Barbour and Rapoport have reported certain observations made by them in 1916. Epinephrin was administered by rectum to rabbits in doses of 1 mgm. per kilo. This resulted in pressor reactions in nearly all cases, the rise of pressure varying from 0 to 28 mm. mercury. The maximum rise appeared in from one-half to about six minutes. Apparently no depressor responses were obtained.

The observations herein recorded were made on four dogs and several cats. In case of one dog the femoral artery was cannulated under local (quinine and urea hydrochlorid) anesthesia and the experiment carried out without general anesthesia. In this instance epinephrin in doses up to 1.6 mgm. by rectum produced no perceptible effect, although the animal gave a clean-cut depressor response to 0.025 mgm. by vein. The other dogs received morphin ($\frac{1}{2}$ grain) and ether throughout the experiment, or paraldehyde by mouth without morphin. The cats were anesthetized with ether in each case. Since there was no apparent difference between the reactions in the cats and those in the dogs, the results may all be discussed together.

In the earlier experiments arterial blood pressure alone was used as an indicator of the response to the drug. This in some cases gave negative results; in the later experiments, therefore, intestinal persistalsis was also recorded, using the Trendelenberg technique. In order to eliminate sympathetic inhibitory impulses, the splanchnic fibers leading to the intestine were cut peripheral to the mesenteric ganglia. Usually, this served to bring about active peristaltic waves. In two instances, however, it proved necessary to ligate off the suprarenal glands before activity began. In order to eliminate oscillations due to respiratory movements, the gut was affixed in two places to a rubber band stretched around the rim of a glass tube 3 cm. in diameter. Stitches through a narrow zone of peritoneal and muscular layers of the gut were taken with silk thread. The thread was then passed beneath the rubber band and tied. From the middle of the segment thus immobilized a thread was led off to a light recording lever. The
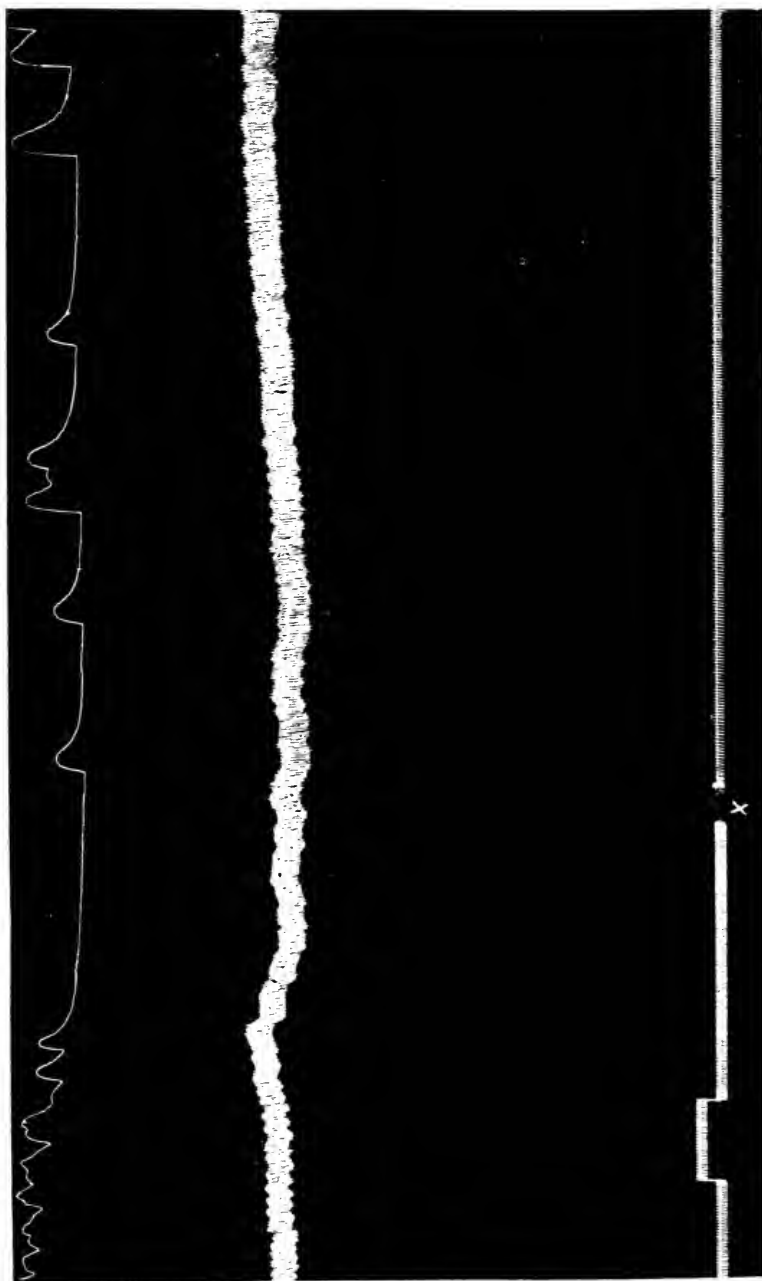
EFFECT OF EPINEPHRIN BY RECTUM ON INTESTINAL PERISTALSIS AND CAROTID BLOOD PRESSURE

Cat, female, weight 2.4 kgn. Ether anesthesia; 1 mgn. epinephrin in 6 cc. water by rectum. Drum stopped two minutes at *x*. Peristalsis stopped for three minutes with slow irregular recovery. Time, one second.

tube was then rigidly clamped in a vertical position to a heavy stand and the abdomen closed with sutures. By this technique a very sensitive preparation for the study of epinephrin and other drugs affecting smooth muscle can readily be secured.

The reactions were found to vary greatly in different animals. In one cat 5 mgm. of epinephrin administered by rectum in three doses at intervals of about two minutes produced only a barely perceptible reaction, a fall in blood pressure. No effect on intestinal peristalsis could be detected. This animal reacted definitely to 0.002 mgm. by vein, both peristalsis and blood pressure being affected. In one dog weighing 6.5 kilos, 4 mgm. at a single dose produced only a slight pressor effect that persisted for about an hour. Since this animal was under paraldehyde anesthesia, the change of blood pressure, although amounting to but 10 mm. of mercury, could rather definitely be ascribed to the epinephrin.

In some animals, however, that were more sensitive to the drug or in which absorption occurred more freely, doses of 1 mgm. produced definite effects. Such a case is illustrated in the accompanying figure. In this subject, a cat weighing 2.4 kilo, blood pressure was but slightly affected; it first rose 5 mm., then fell about the same distance below the normal, gradually returning to the initial level in the course of five minutes. Peristalsis, however, was brought to a complete standstill for three minutes, then, after a series of waves at irregular intervals during the next three minutes resumed its normal course.

The results of the experiments indicate that rectal injections of epinephrin are roughly comparable in quantitative effects to intramuscular injections. Either none or only relatively slight pharmocodynamic reactions were obtained; when secured these persisted from a few minutes to an hour. For studies in pharmacodynamics the method seems too uncertain in its results to be of much value. The data as a whole indicate that absorption does occur, but at a relatively slow rate.

These facts having been determined, further expenditure of time on the quantitative aspects of the problem did not seem warranted.

SUMMARY

Epinephrin was administered to dogs and cats by rectum. Blood pressure and intestinal peristalsis were recorded. The drug, in doses of from 1 to 5 mgm., produced either no or relatively slight effects. In positive reactions peristalsis was depressed and blood pressure either agumented or depressed, the effect persisting from three minutes to an hour in various cases.

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STUDIES ON THE INFLUENCE OF PHENYLCINCHONINIC ACID AND THE ETHYL ESTER OF PARA-METHYLPHENYLCINCHONINIC ACID ON RENAL EXCRETION[1]

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It has been recognized for some time that the administration of salicylates and phenylcinchoninic acid (cinchophen) results in an increased excretion of uric acid in the urine. The influence of phenylcinchoninic acid upon uric acid elimination was first observed by Nicolaier and Dohrn (1) and ascribed by them to a stimulation in nuclear metabolism, but later Weintraud (2) advanced the view that this drug exerts a selective action upon the kidneys, by which uric acid is removed from the blood and tissues. Several years later it was shown by Folin and Lyman (3), and Fine and Chace (4), in particular, that this action is accompanied by a marked drop in the uric acid content of the blood, and later the same was shown to be true of salicylates by Fine and Chace (5), and Denis (6). As a result of the studies on the blood it became clearly evident that the increased elimination of uric acid after the administration of these drugs was due to increased renal function. The action of phlorhizin and the methylated purines on the kidney is better known but scarcely as remarkable as that of the drugs mentioned. The action of phlorhizin holds

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[1] Phenylcinchoninic acid and *p*-methylphenylcinchoninic acid ethyl ester were originally marketed under the names of "atophan" and "novatophan" by their German manufacturers and patentees. In 1919 the Council on Pharmacy and Chemistry of the American Medical Association adopted the nonproprietary name of cinchophen to replace the name atophan and later the name neocinchophen in place of novatophan. The latter product has recently been put on the market under the name of tolysin.

little of direct clinical interest, and Christian and his coworkers (7) have recently thrown serious doubts on the therapeutic value of the methylated purines.

It has been assumed that salicylic acid and cinchophen and their derivatives induce an increased output of uric acid by endowing the renal cells with an increased power for eliminating uric acid. Fine and Chace (8) have pointed out that in the last stages of interstitial nephritis cinchophen has little influence on the excretion of uric acid, indicating that the renal cells can no longer be stimulated to increased activity.

In their experiments Folin and Lyman (3) noted the interesting fact (in two cases) that cinchophen "not only brings about a diminution of the uric acid of the blood but also seems to lead to a diminution of the nonprotein nitrogen and urea whenever these are present in the blood in unusual amounts." Unfortunately these two experiments were not followed by a control after period, making their results less conclusive than they might otherwise have been. Fine and Chace (4) state, "There appears to be no question that 'atophan' increases renal permeability for uric acid and less regularly and to a less extent for other blood constituents." Their work was carried out in this laboratory. No data were given on constituents other than uric acid for the reason that Fine and one of us then contemplated the present study. A year later Denis (6) reported some observations on the action of acetyl salicylic acid and sodium salicylate. She states, "In two or three cases, however, it will be noted that the nonprotein nitrogen is abnormally high and in these cases a considerable diminution of the fraction took place after the administration of the drug." As a result of her observations she concludes,

From the foregoing results the increased output of uric acid following salicylate medication is clearly due to a lowered threshold value of the kidney, not only for uric acid, but in all probability for other waste products as well. Such being the case, it may well be that the beneficial effects resulting from the use of salicylates in acute rheumatic fever may in part at least be due to a power possessed by this class of drugs of increasing kidney permeability, thereby facilitating the rapid and more or less complete excretion of the as yet unknown toxins which produce symptoms of these diseases.

We have endeavored to obtain further light on the mechanism of the action of cinchophen, salicylic acid and their derivatives by experiments upon both man and animals. Studies on the effect of these drugs on the composition of the blood have been made in a series of more than fifty human cases, the larger number being with cinchophen and tolysin. In recent experiments tolysin has more often been employed than cinchophen for the reason that it is tasteless and, being an ester rather than an acid does not give rise to gastric disturbances. Setting aside cases of advanced interstititial nephritis where the action of cinchophen is comparatively slight, we have been struck by the fact that this drug and tolysin both show quite different ability to lower the blood uric acid in different individuals, the action being very pronounced in some cases and comparatively slight in others. In some of these cases an explanation of the degree of renal reaction is not apparent. It may be noted, however, that in arthritic cases who have been in the habit of taking one or another of these drugs, including salicylates, the administration of cinchophen or tolysin may produce comparatively little effect upon the blood uric acid. This is also true in some cases of nephritis with only slight nitrogen retention, while in other cases with a similar degree of retention, these drugs appear to exercise a marked stimulant effect upon the kidney.

For the present discussion six cases have been selected, the first five of which show slight nitrogen retention, and two of which show salt retention. Data are recorded for the uric acid, urea and creatinine of the blood in all cases, for the chlorides in three cases and for the nonprotein nitrogen in one case. The analytical methods employed in the blood analyses were all carried out exactly as described elsewhere by one of us (9).

The data presented on the first three cases shown in table 1 furnish unmistakable evidence, we believe, of a drop in the blood urea as well as in the uric acid, as a result of the administration of both tolysin and cinchophen. Somewhat similar, although possibly less conclusive, results are given on cases 4 and 5 in tables 2 and 3. Strictly normal figures for the urea nitrogen of the blood of the adult when taken in the morning before breakfast

may be given as 12 to 15 mgm. per 100 cc. It will be noted in table 1 that initial figures varying from 20 to 29 mgm. were obtained, while after the administration of the drugs figures varying from 13 to 16 mgm. were found. The discontinuance of the drugs results in a complete or partial restoration of the initial figures.

TABLE 1

Blood level of uric acid, urea and creatinine as influenced by tolysin and cinchophen

CASE AND DIAGNOSIS	DATE 1920–21	BLOOD ANALYSES MGM. TO 100 CC.			DRUG ADMINISTRATION, REMARKS
		Uric acid	Urea N	Creati- nine	
Case 1, M. G., male, aged fifty- eight, chronic in- terstitial nephri- tis, arteriosclero- sis	March 26	6.0	29	1.5	Tolysin 50 grains daily March 28 to April 3
	March 28	5.2	29	2.4	
	March 31*	Trace	16	2.0	
	April 5	3.0	24	2.2	
	April 12	3.5	18	3.0	
	April 13	3.9	15	2.4	
Case 2, M. D., fe- male, aged fifty- three, neurasthe- nia, visceroptosis	March 16	2.8	26	2.6	Tolysin 50 grains daily March 19 to 22. Cinchophen 50 grains daily April 9 to 12. Vegetable diet throughout
	March 18	2.8	22	2.6	
	March 22*	Trace	13	2.7	
	March 25	2.6	20	2.4	
	March 29	2.6	19	2.2	
	April 9	2.8	16	2.4	
	April 12*	0.7	13	2.6	
	April 15	2.0	17	2.4	
Case 3, J. C., fe- male, aged forty, cardionephritis	January 6	7.4	20	3.6	Tolysin 50 grains daily January 9 to 15. Diet con- stant, at rest in bed throughout, Some clinical im- provement noted about January 15
	January 8	7.1	21	3.1	
	January 12*	4.8	15	2.2	
	January 15*	Trace	14	2.5	
	January 20	6.8	23	3.2	
	January 27	6.0	26	3.5	

* Specimens taken during period of medication.

The strictly normal content of uric acid in the blood of the human adult may be taken as 2 to 3 mgm. per 100 cc. All of the first five patients with the exception of case 2 had high initial blood uric acid figures (5 to 7.5 mgm.). These were reduced to a trace in all instances except case 4, where the renal impairment was more pronounced than in the other cases.

Case 3 showed a slight creatinine retention which was favorably influenced by the tolysin administration. With the discontinuance of the drug the initial creatinine figures were restored.

Some of the points in connection with case 4 have already been discussed. As will be noted in table 2, the influence of tolysin was rather more pronounced upon the urea than upon the uric acid. Owing apparently to the severity of the renal involvment, only moderate reduction in the blood uric acid was noted even after rather large doses of this drug had been given. It is significant, however, that even here the drug produced a stimulating rather

TABLE 2

Data illustrating the influence of tolysin on a moderately severe nephritic

DATE 1921	BLOOD ANALYSES MGM. TG 100 CC.					DRUG ADMINISTRATION, (TOLYSIN)
	Nonpro- tein N	Uric acid	Urea N	Creatinine	Chlorides as NaCl	
April 12		5.0	32	2.6	588	50 grains daily April 15–
April 14	60	6.8	38		594	18; 125 grains daily
April 18*	46	5.8	32	2.4	563	April 19–21; after 2
April 21*	49	4.3	23		560	days of 200 grains
April 25*	44	4.8	24	2.2	563	daily
April 30	48	5.8	36	3.6	575	

Case 4, E. G., male, aged twenty-three. Diagnosis, secondary contracted kidney. Patient at rest in bed during whole period of observation. Diet low in protein and no medication other than tolysin. Phenolsulfonephthalein output 30 per cent on April 18. The fluid intake and output were carefully measured from April 13 to 24. On April 17 the urine output equalled the intake and from the 18th to the 24th exceeded the intake by from 100 to 400 cc.

* Specimens taken during period of medication.

than a depressing action on the kidneys, judging from the various blood analyses. The fact that no retention resulted from the rather large dosage would indicate no unfavorable effect on the kidney. Furthermore there appeared to be some clinical improvement.

It will be noted in the data on case 6, table 3, that tolysin in doses of 50 grains per day produced practically no change in the blood uric acid, and only a moderate reduction when the dosage was increased to 100 grains per day. This patient had long taken salicylate compounds, although they had been discontinued some

little time before the present study. The initial uric acid findings were normal, while the urea nitrogen figures are low normals in all cases.

Judging from the data given in the last three cases the administration of cinchophen and tolysin may likewise reduce the concentration of the blood chlorides as well as that of uric acid and urea. Inasmuch as satisfactory after control periods were not obtained in these cases, the findings are hardly as conclusive as in the case of the uric acid and urea, still they appear quite unmis-

TABLE 3

Cases illustrating influence of cinchophen and tolysin on blood chlorides

| CASE AND DIAGNOSIS | DATE 1920–21 | BLOOD ANALYSES MGM. TO 100 CC. | | | | DRUG ADMINISTRATION, REMARKS |
		Uric acid	Urea N	Creatinine	Chlorides as NaCl	
Case 5, A. P., female, aged sixty, chronic arthritis and carcinoma of breast	September 20	5.9	22	1.9	607	Cinchophen 37.5
	September 29	5.6	23	2.0	600	grains per day
	October 3*	2.0	22		488	September 30
	October 6*	2.5	17		450	to October 9.
	October 10*	Trace	19	2.1	475	Low protein diet throughout
Case 6, E. R., female, aged thirty-eight, arthritis deformans	March 7	2.6	11	2.2	508	Tolysin 50 grains
	March 10	2.9	11	2.2	500	daily March 10
	March 13*	2.8	12	2.5	470	to 12 and 100
	March 16	3.4	11	1.8	475	grains March 16
	March 19*	1.4	11	1.5	438	to 18

* Specimens taken during period of medication.

takable. The normal content of chlorides (as NaCl) in human whole blood may be given as 450 to 500 mgm. per 100 cc. Cases 4 and 5 both showed high initial figures, while with case 6 they were essentially normal. Still in this case the reduction in the chloride concentration is quite definite.

Cinchophen and tolysin, as well as the salicylates, possess marked analgesic properties. Various suggestions have been offered to correlate this effect with their action on the kidney, but no data are available which throw much light on this question. It is well known clinically that without increasing the doses of

these drugs their analgesic effect is gradually lost. Consequently they are generally administered in rather large doses for short periods. Similarly their stimulant action on uric acid excretion is gradually lost with their continued administration.

To secure more light on the mechanism of their action on the kidney, a large number of experiments have been carried out in this laboratory on animals by Drs. Simpson and Meeker (10). Their observations, as yet unpublished, suggest that the administration of this group of drugs in rather large doses results in congestion and irritation of the glomeruli, in particular, the changes being, for the most part, mild in degree. These findings afford a possible explanation of the improved renal function noted after the administration of cinchophen, salicylic acid and their derivatives. Assuming that this improved renal function is dependent upon an altered blood supply, it seems quite possible that the beneficial effects in arthritis may be the result of a similar alteration of the blood supply to the affected areas.

As pointed out by Mosenthal and Lewis (11), and others, some cases of chronic diffuse nephritis and hypersensitive cardiovascular disease show a superpermeability of the kidney, i.e., low blood ureas, high phenolsulfonephthalein outputs, etc. These cases of overactivity in mild kidney disease have been attributed to the irritation resulting from inflammatory processes. As the lesion advances the renal parenchyma becomes more severely damaged and then exhibits a subnormal activity to the pathologic stimulus. These findings appear to be analogous in certain respects to the results obtained by the administration of salicylates, cinchophen and their derivatives.

In previous communications (12) we have called attention to the fact that of the three nitrogenous waste products, uric acid, urea and creatinine, creatinine is the most readily and uric acid the most difficultly eliminated, with urea standing in somewhat of an intermediate position. In harmony with this a uric acid retention may be found in the early stages of nephritis, but creatinine retention only in the terminal stages of the disease. One would therefore expect that drugs, which had a general stimulating effect on the kidney, would affect the excretion of uric acid first, urea

next and creatinine last. From the observations recorded in table 1 such appears to be the case. This would indicate that the action of these drugs is not *specific* for uric acid. To be sure, the action on the uric acid is by far the most marked, but the term "uric acid eliminant" frequently applied to these drugs is rather misleading.

SUMMARY

Phenylcinchoninic acid (cinchophen) and the ethyl ester of *p*-methylphenylcinchoninic acid (tolysin) exercise a general stimulating effect on kidney excretion. This action is most marked in the case of uric acid, but it is possible to demonstrate a similar action in the case of urea and chlorides, provided cases are selected with a slightly high blood concentration of these substance.

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THE EFFECT OF WATER DIURESIS ON THE ELIMINATION OF CERTAIN URINARY CONSTITUENTS

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The diuresis produced by the ingestion of large quantities of water by mouth has frequently been made the subject of investigation. From the standpoint of the effect of the increased urine flow on the rate of elimination of the various solid constituents of the urine very little accurate data is available. Investigators have usually taken twenty-four hour periods of collection (which may mask the changes produced by diuresis) or have not sufficiently controlled their experiments. The general idea appears to be that the absolute amount of all the solids is increased while the percentage of all falls (1). The recent paper by Marshall (2) on the influence of water diuresis on the elimination of urea, creatinine, and chloride in the dog and man contains a brief summary of the earlier work.

The work of Marshall and Kolls (3) and the recent work of Marshall and Crane (4) indicate that with increased blood flow through the kidney the elimination of water, chlorides and carbonates is markedly increased; that of urea, sulphates, phosphates increased but to a less extent, while that of creatinine, ammonia, and phenolsulphonephthalein is not influenced.

Marshall (2) has found that with water diuresis "creatinine is not increased to a measurable extent; urea is increased definitely but not more than two fold; chlorides are apparently increased, but the increase is variable and generally less marked than that of urea." The present work was undertaken to extend this investigation to other substances which had been studied in regard to the effects of blood flow.

The effect of increased urine flow caused by the ingestion of water on the elimination of chlorides, carbonates, sulphates, phosphates, urea, creatinine, ammonia, and hydrogen ion concentration of the urine was studied in normal men. The determinations of urea, creatinine, and chloride were included simply to correlate the work with that of Marshall, and the creatinine also served as a convenient check on complete collection of samples. The data obtained confirm in all respects the earlier work. Complete control experiments without the ingestion of water but otherwise under as nearly identical conditions as possible were also carried out the days preceding or immediately following the diuresis experiment. The importance of this is well illustrated by the behavior of the phosphates. As is shown here and has previously been shown by Fiske (5), Leathes (6) and Broadhurst and Leathes (7), the phosphate excretion follows a curve decreasing in the morning hours and increasing in the afternoon. If a normal control had not been run it would have been concluded that diuresis caused a definite decrease or increase in phosphates, depending on whether the experiment had been performed in the morning or afternoon.

METHODS

All the experiments were carried out on normal men. A control experiment was performed in all cases without the ingestion of water, except as shown in the protocols, but otherwise under similar conditions as the diuresis experiment. All subjects were given 200 to 250 cc. of water one to two hours before the collection of samples was begun. In the morning experiments no food had been taken since 7 to 8 p.m. the preceding evening. For the afternoon experiments the noon meal was omitted.

After the urine for the carbonate and hydrogen ion concentration had been measured and removed, the samples were diluted. The samples during diuresis were usually analyzed undiluted, but the concentrated sample of that day was diluted to about the same volume. Determinations of creatinine, ammonia, urea, sulphates, phosphates, chlorides, carbonates, and the hydrogen ion concentration were performed on each sample. Creatinine

was determined by Folin's method (8), urea by the urease method (9), ammonia by the aeration method (10), sulphates by the benzidene method (11), phosphates by Bell and Doisy's colorimetric method (12), chlorides by the Volhard method, carbonates, i.e., the total carbon dioxide content, by Van Slyke's method (13), and hydrogen ion concentration by the colorimetric method of Henderson and Palmer (14) using the Lubs and Clark indicators (15).

It was realized after about five or six experiments had been performed that extreme precautions were necessary for the accurate determination of the carbonates and the hydrogen ion concentration of the urine. The subjects were instructed to urinate toward the side of the cylinder with as little bumping as possible. The specimen of urine (5 cc.) was immediately transferred to the Van Slyke apparatus. This procedure never requires over one minute. A double extraction and absorption of the carbon dioxide with alkali was always done. The hydrogen ion concentration must also be determined at once and with as little shaking as possible, especially in the dilute urines. By this method it is possible to determine 90 to 95 per cent of the carbonates present in the urine. In a number of experiments in which the urine was collected without exposure to air, and then allowed to drop through air, and to slide down the side of a cylinder from a separatory funnel in which the collection had been made without exposure to air, the loss of carbon dioxide was from 5 to 8 per cent for a P_H range of 5.10 to 7.00.

Agitation, such as shaking or pouring, of a sample of urine before determination of the hydrogen ion concentration may lead to highly erroneous determinations; especially is this true of the urines with a high carbonate content or with very dilute urines.

RESULTS

The results are presented in detail in the tables in the appendix (tables 6 to 17). Due to the considerable variations in a number of the constituents on control days and the rather slight effects produced by diuresis, a different form of tabulation of the results is presented here. All the data on each constituent is collected

in one table. The first period (the first hour) is taken as 100 in both the control and diuresis days of the experiment, and amounts present in the subsequent periods are represented in percentages of the first period.

Thus in table 1 the results for urea indicate that there is a distinct tendency for this substance to decrease on the control days from hour to hour, but this finding is not constant (experiments 6, 7, 8, 10 and 12). The amount of urea eliminated is increased in the second period on the diuresis days (first hour of the diuresis) in all instances except experiment 10, and is also usually increased in the third period. In experiment 10, it is

TABLE 1

Urea

EXPERIMENT NUMBER	CONTROL				DIURESIS			
1	100	91	86	58	100	130	136	107
2	100	93	85	71	100	166	151	133
3	100	94	79	68	100	100	92	83
4	100	90	81	82	100	122	93	68
5	100	89	65	56	100	127	84	76
6	100	85	102	96	100	177	213	151
7	100	127	96	82	100	161	133	101
8	100	97	104	79	100	140	91	118
9	100	85	82	85	100	132	139	85
10	100	123	94	82	100	123	140	102
11	100	85	88	75	100	132	121	91
12	100	146	115	118	100	194	227	140

increased in the third period. Urea, then, is apparently always increased by water diuresis.

As has been mentioned the results obtained in this investigation as to creatinine, urea, and chlorides correspond in all respects to those obtained by Marshall (2).

In table 2 the data on sulphates are collected. The same general tendency for the elimination to decrease in the morning hours is seen, as with the urea. This is apparently independent of the water elimination. In nine out of the twelve experiments sulphates are increased in the second and frequently in the third hour of the diuresis experiment. In experiments 7 and 10 the

increase is doubtful, while in experiment 8 there is no increase. Taken as a whole the experiments indicate a distinct increase in the elimination of sulphates during water diuresis.

Table 3 gives the data on phosphates. With the exception of experiments 9 and 12, the phosphates exhibit a marked decrease during the morning hours (experiments 1, 2, 3, 4, 5, 6, 10) and an increase during the afternoon hours (experiments 7, 8 and 11) as has been noted by previous investigators. The variations in phosphates on the control days is much greater than that of any of the other consistuents and the interpretation of the effect of diuresis is more difficult. In eight out of the twelve experiments

TABLE 2

Sulphates

EXPERIMENT NUMBER	CONTROL				DIURESIS			
1	100	72	74	60	100	103	157	109
2	100	80	68	66	100	131	165	167
3	100	91	72	70	100	96	110	100
4	100	76	75	73	100	107	84	69
5	100	83	74	59	100	97	71	72
6	100	95	87	87	100	102	115	86
7	100	110	108	101	100	115	91	86
8	100	111	110	88	100	113	81	108
9	100	71	73	64	100	151	169	134
10	100	101	82	68	100	90	107	80
11	100	93	96	81	100	118	138	109
12	100	119	90	80	100	130	210	131

there is an increase during diuresis in the second and third periods or both as compared to the control days (experiments 1, 2, 3, 5, 9, 10, 11, 12). In seven other experiments of similar nature, but not included because of incompleteness, the phosphates were increased in five.

An examination of table 4 shows that ammonia is frequently unchanged during diuresis (experiments 1, 3, 5, 6, 7) but may be increased in the second or third periods. In seven other experiments not included an increase in the second or third periods or both was noted in six experiments. Consequently the ammonia appears to be more often increased during water diuresis than unchanged.

TABLE 3

Phosphates

EXPERIMENT NUMBER	CONTROL				DIURESIS			
1	100	67	67	64	100	83	103	68
2	100	47	43	43	100	78	67	59
3	100	67	60	72	100	77	72	62
4	100	55	49	48	100	52	39	34
5	100	50	36	31	100	78	42	42
6	100	93	56	86	100	74	65	62
7	100	151	146	98	100	126	102	77
8	100	133	150	106	100	110	93	114
9	100	87	94	78	100	126	136	89
10	100	88	67	64	100	85	157	118
11	100	122	110	100	100	136	152	98
12	100	98	102	104	100	107	136	117

TABLE 4

Ammonia

EXPERIMENT NUMBER	CONTROL				DIURESIS			
1	100	113	110	82	100	98	93	91
2	100	93	82	56	100	130	84	98
3	100	99	75	92	100	91	82	77
4	100	88	67	56	100	114	110	59
5	100	88	83	55	100	99	52	70
6	100	82	73	51	100	77	86	82
7	100	100	96	112	100	102	102	96
8	100	110	79	90	100	110	101	104
9	100	99	113	104	100	128	68	137
10	100	103	79	66	100	93	143	107
11	100	89	86	83	100	156	253	158
12	100	95	84	78	100	134	177	136

TABLE 5

Per cent carbon dioxide

PERIOD	EXPERIMENT NUMBER						
	6	7	8	9	10	11	12
1	0.007	0.006	0.007	0.007	0.011	0.036	0.012
2	0.008	0.008	0.010	0.011	0.023	0.020	0.031
3	0.012	0.009	0.010	0.010	0.020	0.014	0.023
4	0.010	0.009	0.009	0.007	0.014	0.008	0.021

Creatinine, chlorides, urea, sulphates, phosphates, and ammonia are all markedly decreased in percentage during diuresis. The data for carbonates (contained in tables 11 to 17) indicate so clearly that these are markedly increased during diuresis that no special table is necessary. The carbonates may be increased even more than the water, e.g., in experiment 6 the water is increased twenty-five fold and the carbonates over forty times. Table 5 indicates that there is generally an increase also in the percentage of carbon dioxide during diuresis, as well as the absolute amount.

During diuresis there was a decrease in hydrogen ion concentration in all experiments except experiment 11. On the control days there was considerable variation in the hydrogen ion concentration, frequently however becoming more acid as the experiment progressed.

<div style="text-align:center">DISCUSSION</div>

Although the experiments were planned to be carried out under as nearly the same conditions as possible on the control and diuresis days, it is obvious from the great variations which occur from hour to hour even on the control days that a great many uncontrolled factors were present. Whether these are variations in the activity of the kidneys or variations caused by changes in the concentration of substances in the blood cannot be decided without blood analyses for each constituent in each period of the experiments. This was impossible to do during the present work, which must, therefore, be considered as a preliminary survey of the question of the influence of water diuresis on the constituents of the urine. The effects of posture, muscular activity, etc., of the subject were probably not sufficiently controlled, and a further control of interfering factors could probably be obtained by keeping the subject in bed in a quiet room during all the experiments.

Certain effects of water diuresis, however, seem to be so definite that in a long series of experiments they are obvious in spite of the interfering factors. The general current idea that all the constituents of the urine are decreased in percentage but increased in absolute amount is not borne out by these experiments. The carbon dioxide is generally increased in percentage as well as in

absolute amount. The creatinine is unchanged by diuresis. Urea and sulphates are consistently increased in absolute amount although the increases do not appear to be parallel. The phosphates are seen to be frequently unchanged but more often seem to be slightly increased. In view of these experiments the results of Bock (16) who found no change in the elimination of phosphates in rabbits during water diuresis, as well as those of Baetzner (17) who found under the same conditions increases of several hundred per cent do not appear to be of much value as proper control experiments without diuresis were not done. The phosphates then, generally are slightly increased during water diuresis, although they may be unchanged. This probably does not mean a variation in the activity of the kidney towards phosphates during diuresis, but is probably due to the interfering factors such as blood concentration, etc. Moreover, the fact that the excretion of phosphates being one of the main factors in regulating the acid-base equilibrium of the body will probably have to be taken into account. The excretion of ammonia is also a well known means of neutralizing acid and eliminating it from the body, and the excretion of ammonia has been shown beyond question to depend on the state of the acid-base equilibrium of the body (18). It is not, therefore, surprising that variable results are obtained. However, in the majority of cases the elimination of ammonia is increased during diuresis. This may, however, be due to the fact that since the alkalinity of the urine is increased during diuresis, and there is presumably a slightly greater acidity of the body, that this is responsible for the increase in ammonia. The statement in a recent paper by Calvert, Mayrs and Milroy (19) that the amount of ammonia eliminated is unchanged by water diuresis is based on one experiment and is found not to be true when several experiments are carried out.

In general the hydrogen ion concentration is decreased during water diuresis. It has repeatedly been observed that the urine becomes more alkaline during diuresis (20). The statement made recently by Leathes (21) that in subjects without food for twelve to eighteen hours, there is a definite alkaline tide in the morning hours independent of diuresis is found not to be universally true in these experiments.

Although in a number of the experiments different amounts of fluid were taken to produce diuresis, the results are not sufficiently constant to determine whether the extent of the increase in the solid constitutents is dependent on the extent of the diuresis. In general it appears that very large amounts of fluid cause no greater increases than smaller ones.

SUMMARY

The effect of water diuresis on the elimination of chlorides, creatinine, urea, ammonia, sulphates, phosphates, and carbonates has been studied in normal human subjects. The data obtained confirm the finding of Marshall in regard to urea, creatinine, and chlorides. Sulphates are increased in practically all experiments. Ammonia and phosphates are generally increased, but may be unchanged. All of these substances are decreased in percentage. Carbonates (i. e., total carbon dioxide) are increased not only in absolute but in percentage amount. The hydrogen ion concentration of the urine is decreased during diuresis.

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APPENDIX

TABLE 6

Subject A. D. C.

TIME	VOL-UME (cc.)	CREATININE (mgm.)	NH4 (mgm.)	UREA (mgm.)	SO4 (mgm.)	Cl (mgm.)	PO4 (mgm.)
8:00– 9:00	45	61	43	900	90	180	60
9:00–10:00	414	58	42	1174	92	342	50
10:00–11:00	950	59	40	1232	141	402	62
11:00–12:00	610	55	39	964	98	147	41

9:05–9:15   drank 3000 cc. water.

TIME	VOL-UME (cc.)	CREATININE (mgm.)	NH4 (mgm.)	UREA (mgm.)	SO4 (mgm.)	Cl (mgm.)	PO4 (mgm.)
8:20– 9:20	66	58	32	867	68	388	93
9:20–10:20	126	55	37	793	50	293	63
10:20–11:20	117	55	36	751	51	272	62
11:20–12:20	35	56	27	502	41	202	60

TABLE 7

Subject A. D. C.

TIME	VOL-UME (cc.)	CREATININE (mgm.)	NH4 (mgm.)	UREA (mgm.)	SO4 (mgm.)	Cl (mgm.)	PO4 (mgm.)
7:55– 8:55	20	55	21	469	40	59	46
8:55– 9:55	475	55	28	781	52	173	35
9:55–10:55	629	52	18	708	65	123	30
10:55–11:55	440	50	21	624	66	941	27

9:00–9:10   drank 2000 cc. water.

TIME	VOL-UME (cc.)	CREATININE (mgm.)	NH4 (mgm.)	UREA (mgm.)	SO4 (mgm.)	Cl (mgm.)	PO4 (mgm.)
7:55– 8:55	37	58	32	557	40	60	83
8:55– 9:55	84	52	30	523	32	84	40
9:55–10:55	53	55	26	487	27	92	36
10:55–11:55	26	53	18	397	27	107	36

9:00–9:10   drank 2000 cc. water.

TABLE 8

Subject A. D. C.

TIME	VOLUME	CREATININE	NH₄	UREA	SO₄	CL	PO₄
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
7:50– 8:50	81	60	33	885	63	286	68
8:50– 9:50	442	63	31	889	61	395	52
9:50–10:50	640	60	28	821	70	221	49
10:50–11:50	445	59	26	737	63	110	42

8:55–9:00   drank 2000 cc. water.

TIME	VOLUME	CREATININE	NH₄	UREA	SO₄	CL	PO₄
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
8:00– 9:00	60	53	30	603	40	241	48
9:00–10:00	66	50	30	568	36	257	31
10:00–11:00	45	50	23	480	30	151	27
11:00–12:00	30	53	28	412	29	140	35

TABLE 9

Subject A. D. C.

TIME	VOLUME	CREATININE	NH₄	UREA	SO₄	CL	PO₄
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
7:55– 8:55	35	62	30	540	39	98	52
8:55– 9:55	260	60	35	651	42	187	27
9:55–10:55	196	53	34	495	33	135	20
10:55–11:55	36	52	18	361	27	90	18

9:00–9:02   drank 500 cc. water.

TIME	VOLUME	CREATININE	NH₄	UREA	SO₄	CL	PO₄
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
8:10– 9:10	39	55	33	564	47	131	86
9:10–10:10	47	54	29	508	36	155	48
10:10–11:10	50	51	23	456	35	189	42
11:10–12:10	33	50	19	468	34	169	42

TABLE 10

Subject A. D. C.

TIME	VOL-UME	CREA-TININE	NH4	UREA	SO4	Cl	PO4
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
8:00– 9:00	135	52	42	486	40	102	63
9:00–10:00	355	55	42	622	39	194	50
10:00–11:00	260	52	22	412	28	133	27
11:00–12:00	31	54	30	373	29	135	27

9:03–9:05 drank 500 cc. water.

TIME	VOL-UME	CREAT-ININE	NH4	UREA	SO4	Cl	PO4
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
8:00– 9:00	67	54	38	715	51	405	73
9:00–10:00	112	55	34	644	42	497	37
10:00–11:00	76	51	32	470	36	338	26
11:00–12:00	31	54	21	404	30	205	23

TABLE 11

Subject E. K. M.

TIME	VOL-UME	CRE-ATI-NINE	NH4	UREA	SO4	Cl	PO4	CO2	PH
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
8:15– 9:15	20	59	38	580	58	125	49	1.5	5.15
9:15–10:15	57	59	30	1028	59	191	37	4.9	5.20
10:15–11:15	492	60	34	1237	67	229	32	62.0	5.80
11:15–12:15	195	54	32	877	50	158	31	21.0	5.90

9:18–9:22 drank 1000 cc. water.

TIME	VOL-UME	CRE-ATI-NINE	NH4	UREA	SO4	Cl	PO4	CO2	PH
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
8:35– 9:35	29	57	31	737	59	164	44	2.4	5.37
9:35–10:35	28	59	25	630	56	175	42	2.4	5.15
10:35–11:35	25	56	22	669	52	172	25	2.1	5.10
11:35–12:35	29	60	16	712	51	215	33	9.2	6.60

TABLE 12

Subject J. W. W.

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH₄ mgm.	UREA mgm.	SO₄ mgm.	Cl mgm.	PO₄ mgm.	CO₂ mgm.	P_H
1:00-2:00	27	59	30	548	58	288	52	3.3	5.30
2:00-3:06	27	63	30	698	65	186	79	1.8	5.05
3:06-4:06	18	63	29	530	63	157	77	1.0	5.10
4:06-5:09	17	61	34	450	59	140	51	1.0	5.10

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH₄ mgm.	UREA mgm.	SO₄ mgm.	Cl mgm.	PO₄ mgm.	CO₂ mgm.	P_H
1:00-1:55	22	56	29	701	65	213	62	1.5	5.30
1:55-3:08	120	62	30	1134	75	232	79	10.0	5.40
3:08-4:08	355	57	31	935	60	137	64	32.0	5.85
4:08-5:08	42	57	28	713	56	97	48	3.8	5.30

1:56-2:06 drunk 1000 cc. water.

TABLE 13

Subject J. W. W.

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH₄ mgm.	UREA mgm.	SO₄ mgm.	Cl mgm.	PO₄ mgm.	CO₂ mgm.	P_H
1:00-2:00	36	58	23	611	57	434	32	6.2	5.90
2:00-3:00	26	59	26	597	64	277	43	1.8	5.30
3:00-4:00	28	59	19	622	64	314	49	2.2	5.50
4:00-5:00	22	62	21	487	54	265	35	1.6	5.20

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH₄ mgm.	UREA mgm.	SO₄ mgm.	Cl mgm.	PO₄ mgm.	CO₂ mgm.	P_H
12:55-1:55	30	61	31	817	61	360	41	2.2	5.15
1:55-2:55	135	61	35	1147	69	314	46	14.0	5.45
2:55-3:55	420	51	32	751	50	140	38	43.0	5.60
3:55-4:55	480	59	33	971	66	157	47	47.0	6.10

2:00-2:15 drunk 1500 cc. water.

TABLE 14
Subject A. D. C.

TIME	VOL-UME	CRE-ATI-NINE	NH4	UREA	SO4	Cl	PO4	CO2	PH
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
12:00–1:00	20	52	21	522	43	63	51	1.5	5.40
1:00–2:00	118	52	26	690	65	90	64	14.0	5.50
2:00–3:00	350	56	35	730	73	63	70	37.0	5.75
3:00–4:00	156	38	28	446	58	59	46	12.0	5.50

1:03–1:06 drank 1000 cc. water.

TIME	VOL-UME	CRE-ATI-NINE	NH4	UREA	SO4	Cl	PO4	CO2	PH
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
12:00–1:00	51	53	23	681	55	297	67	5.5	5.90
1:00–2:00.	33	53	22	596	39	165	58	2.7	5.60
2:00–3:00	29	57	25	579	40	140	63	2.1	5.50
3:00–4:00	22	53	24	598	35	81	52	1.7	5.45

Drank 50 cc. water every hour.

TABLE 15
Subject C. C. H.

TIME	VOL-UME	CRE-ATI-NINE	NH4	UREA	SO4	Cl	PO4	CO2	PH
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
8:00– 9:00	34	83	43	703	64	426	46	4.0	5.70
9:00–10:00	59	81	40	869	58	351	42	14.0	6.10
10:00–11:00	570	83	62	985	68	373	77	115.0	6.30
11:00–12:00	170	80	46	719	52	260	59	25.0	6.00

9:02– 9:05 drank 1000 cc. water.

TIME	VOL-UME	CRE-ATI-NINE	NH4	UREA	SO4	Cl	PO4	CO2	PH
	cc.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
8:00– 9:00	33	80	48	900	72	288	76	3.2	5.80
9:00–10:00	66	79	50	1109	73	540	67	9.4	6.10
10:00–11:00	62	77	38	852	59	612	51	13.0	6.20
11:00–12:00	50	74	32	743	49	549	49	12.0	6.20

Drank 50 cc. water every hour.

TABLE 16
Subject L. J. E.

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH4 mgm.	UREA mgm.	SO4 mgm.	Cl mgm.	PO4 mgm.	CO2 mgm.	P_H
10:55-11:55	68	69	42	1058	80	517	107	5.4	5.30
11:55-12:55	48	71	38	905	75	360	131	3.8	5.10
12:55- 1:55	39	73	37	939	77	261	118	3.2	5.10
1:55- 2:55	32	68	35	800	65	139	107	2.6	5.00

Drunk 50 cc. water every hour.

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH4 mgm.	UREA mgm.	SO4 mgm.	Cl mgm.	PO4 mgm.	CO2 mgm.	P_H
10:55-11:55	49	64	18	668	46	297	77	18.0	6.60
11:55-12:55	276	71	28	884	51	290	105	56.0	6.15
12:55- 1:55	780	78	46	813	63	257	117	116.0	6.05
1:55- 2:55	59	75	28	609	50	171	75	5.3	5.70

11:00-11:10 drunk 1000 cc. water.

TABLE 17
Subject C. C. H.

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH4 mgm.	UREA mgm.	SO4 mgm.	Cl mgm.	PO4 mgm.	CO2 mgm.	P_H
8:30- 9:30	17	86	23	509	58	117	43	3.9	6.30
9:30-10:30	36	83	22	749	69	270	43	45.0	7.10
10:30-11:30	28	81	19	589	52	207	44	17.0	6.90
11:30-12:30	23	79	18	605	49	152	45	9.4	6.65

Drunk 50 cc. water every hour.

TIME	VOL-UME cc.	CRE-ATI-NINE mgm.	NH4 mgm.	UREA mgm.	SO4 mgm.	Cl mgm.	PO4 mgm.	CO2 mgm.	P_H
8:30- 9:30	21	67	31	535	41	173	48	2.6	5.90
9:30-10:30	138	80	42	1040	51	327	52	43.0	6.50
10:30-11:30	623	77	56	1216	87	382	66	147.0	6.50
11:30-12:30	212	66	43	754	55	379	56	45.0	6.50

9:30- 9:33 drunk 1000 cc. water.

A SIMPLE METHOD FOR THE DETERMINATION OF THE COAGULATION TIME OF BLOOD IN ANIMALS

O. INCHLEY

From the Pharmacological Laboratory, Cambridge

Most observers agree that the determination of clotting time in animals, when small quantities of blood are used, gives very variable results. I have tried various methods which can be used with a single drop of rabbit's blood and have obtained results so variable that no deduction could be drawn from them.

The method which was ultimately adopted and which has given consistent readings, is a modification of Buckmaster's (1) and consists essentially in taking up a drop of blood in a fine wire loop and observing when, on slowly rotating the loop by hand, the blood no longer moves freely under gravity. It is convenient to note the time when the first sign of altered mobility appears, and also when complete fixation of the drop occurs. The mean of these is taken as the end of clotting time. I think this preferable to fixing on one arbitrarily chosen end point. The preparation is maintained at blood temperature in a small moist chamber.

The apparatus consists of a loop 5 mm. in diameter, made of fine nickel wire and shaped as in figure 1. It is easily made by taking two lengths of wire fixed at one end, inserting between them a glass rod almost 5 mm. in diameter and twisting. After shaping as in the figure, the wire is gently hammered on an anvil to remove spring. The moist chamber is composed of two glass cells, used for microscopic purposes, which can be ringed with plasticene and pressed together so as to form a small air chamber. The preparation is inserted in this air chamber and held by a clamp which also serves for rotating (see figs. 1 and 2). The observation is made under water at 37° to 40°C. in sufficient

237

quantity to maintain an approximately even temperature during the experiment, and it is convenient to employ a submerged lamp and a reflecting mirror inclined at 45 degrees. The temperature of the water required is most quickly attained by adding one volume of boiling water to two of tap water and adjusting.

During an observation the blood is at first so mobile that it falls as quickly as the loop is rotated so that there is no movement to be seen when held vertically. Then comparatively suddenly it becomes thicker, the movement is not completed when the vertical position is attained, so that for a time the whole circle
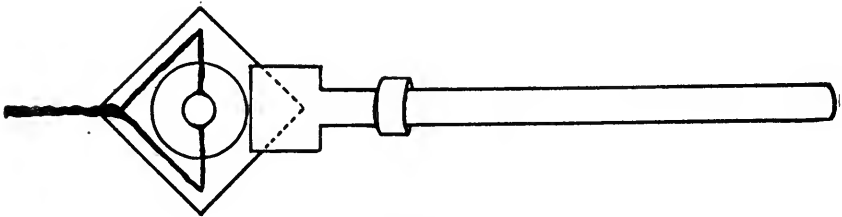
Fig. 1

Fig. 2

is obscured by the falling blood. Finally there is a complete absence of any perceptible movement and the film appears fixed.

The precautions necessary in working this method are as follows:

1. The wire loop must be wetted by the surface of the blood from the vein, and then lifted directly away from it so as to obtain a constant and sufficient amount of blood.

2. The wire extensions from the loop must not be wetted, otherwise the blood will run along them to the walls of the moist chamber.

3. Constant conditions must be aimed at when drawing a drop of blood from the ear vein; unless the blood wells up freely from the puncture it is useless to attempt to make consistent estimations. It is advisable to wipe away the first few drops yielded.

4. The wire loop should be heated in the bunsen before each observation.

5. The two halves of the moist chamber should be kept immersed in the water at 40°C. when not in use. When required, all that is necessary is to shake out the excess of water.

The following are figures obtained during two experiments on rabbits, anesthetized with urethane, before and after treatment with calcium introduced by the "ionizing" current. Blood obtained from ear vein. Time in seconds.

Experiment 1

TIME	TEMPERATURE OF WATER BATH	CLOT COMMENCING	CLOT COMPLETE	MEAN CLOTTING TIME
p.m.	°C.			
2.35	39.0	?	100	100−
2.45	39.0	90	110	100
2.55	39.0	95	113	104
3.00*				
3.35	37.0	?	70	70−
3.40	38.0	59	73	66
4.10	38.0	40	50	45
4.15	38.5	45	52	48

Experiment 2

3.20	38.0	151	180	165
3.30	38.0	174	192	183
3.40	38.0	187	208	197
3.50	37.0	201	217	209
4.10*				
4.40	39.0	275	294	284
4.50	38.0	90	108	99
4.55	37.0	94	111	102
5.20	36.0	105	132	118
5.24	40.0	70	81	75
5.28	40.0	71	87	79
5.32	39.0	78	94	86

* Calcium treatment commenced.

DISCUSSION

Dale and Laidlaw (2) showed that with temperatures approximating to that of the normal blood the time of coagulation was not appreciably affected by small variations, while in the region of room temperature, slight variations greatly affected the clotting time. It is obvious that with any simple apparatus the selected temperature must be about 37° to 40°C. and the apparatus must be small and the heating water large in amount to attain the object in view.

Addis (3) criticized Buckmaster's method favourably except for. the difficulty he experienced in keeping the temperature constant and the difficulty of obtaining thin blood films of constant thickness, the movement of the corpuscles being observed with a lens in this method. In the present method the maximum quantity of blood is picked up in the wire circle by lifting it vertically away from the pool of blood; it is well known that if a wire loop be lifted at various inclinations variable quantities will be retained therein.

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THE INFLUENCE OF THE ELECTRIC CURRENT ON THE ABSORPTION OF DRUGS

O. INCHLEY[1]

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The introduction of drugs into the animal body by means of the electric current has been matter of discussion for about fifty years. But an examination of the literature shows that precise records of experiments are few. Leduc (1) states that by means of the electric current strychnine applied to the rabbit's ear may be forced into the body in sufficient amount to cause convulsions; he discusses also absorption of ions in general though without giving experimental details. Finzi (2) showed that in the anesthetized monkey he could by means of a current of 10 milliamperes applied for thirty minutes obtain some absorption of the ferricyanide ion, which penetrated right into the knee-joint.

The present investigation was undertaken to determine within what limits the electric current facilitated absorption from the skin. Experiments were performed principally on living animals, cats, guinea-pigs and rabbits. The animals were anesthetized with urethane whereby a constant type of blood pressure and respiration can be obtained for some hours. In some cases the skin to which the electrode was applied for the purpose of conveying in the drug was prepared a day or two previously either by shaving the part free from hair, or by destroying the hair with barium sulphide. In other cases the hair was left intact, being simply wetted with the solution of the drug at the time of the experiment. No appreciable difference was noted whichever procedure was adopted. The current amperage and the area of the skin to which it was applied was always noted, and the

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[1] Undertaken during the tenure of a British Medical Association scholarship.

current was kept constant throughout the experiment. The voltage was gradually raised or lowered when required, and as far as possible all sudden alterations were avoided. For application to the skin of the abdomen it was found convenient to use an area of 20 sq. cm. and 4 sq. cm. for the knee-joint, and the current employed was 1 or 2 m.a. per square centimeter.

In order to obtain a continuous ionization with the drug under consideration, and to avoid the irritation due to the acid or alkali liberated at the positive and negative poles respectively, the electrodes used were made as follows: An ordinary porous pot from a battery was filled with a 1 per cent solution of sodium chloride in tap water and in this was placed a carbon rod connected with one pole of the current supply. One or more layers of lint soaked in a 1 per cent solution of the drug in distilled water was inserted between the pot and the skin. The lint here is not essential and the intact fur of the animal similarly soaked with the solution may take its place. In other cases it was convenient to use a piece of lamp-wick soaked with the solution and attached to the pot by a rubber band to convey the current to the part of the animal required. The two poles were always placed at some distance away from each other on the animal, if one pole was on the abdomen the other was often on the leg.

The second pole consisted either of a needle which was inserted into a muscle, or of a second porous pot arranged like the first with the exception that the lint was soaked in a 1 per cent solution of sodium chloride in tap water. Between the lint and the skin a sheet of rubber was placed containing a window of known area so that any passage of current through the skin could only occur through the window.

Blood pressure when necessary was taken from the carotid artery, and respiration was recorded from a tambour placed on the sternum. Blood-coagulation time was obtained when required by drawing a single drop of blood from an ear-vein and estimating by a method described elsewhere (3).

A number of experiments have been performed also on dead tissues, and on various isolated organs.

Alkaloids

Atropine.   Under the influence of the electric current most alkaloids are very easily absorbed through the skin of animals into the general circulation.   If 10 sq. cm. of the skin of the abdomen of a cat or rabbit are soaked with a 1 per cent solution of atropine sulphate connected with the negative terminal of a supply yielding a current of 20 m.a., no signs of atropine absorption can be detected in three hours.   That is to say the response of the chorda tympani and vagi to electrical stimulation remains normal, and the pupils contract on exciting the third nerve.   If however the current be reversed in the case of the cat the animal is almost atropinized in five minutes and completely atropinized in ten minutes: in the rabbit the time required to atropinize is somewhat longer.   Animals so treated are uninfluenced by the injection of a dose of pilocarpine or arecoline.

Experiment 1.   Cat, urethane; blood pressure from carotid.   Vagi exposed.   Either vagus stimulated by faradic current, secondary coil at 15 c. produced marked cardiac inhibition.   The electrode described above was placed over the center of the abdomen and connected with the positive pole of the source of current.   The lint here was soaked in a 1 per cent solution of atropine sulphate.

The negative electrode consisted of a steel needle inserted in a thigh muscle.

The area of the positive electrode measured 20 sq. cm.

A current of 5 m.a. was now passed (0.25 m.a. per square centimeters). The vagi ceased to inhibit the heart in ten minutes, even when the secondary coil was at 5 cm.

One cubic centimeter of 0.1 per cent arecoline injected into the jugular vein produced no effect on the rate of the heart, on salivary secretion, or on the pupil.

Atropine solution may be applied to the skin for many hours without any detectable absorption taking place provided no current is passing or if the current be passing, provided the atropine be on the negative pole.   This fact is brought out by the next experiment and verifies Leduc's experiment with strychnine.

Experiment 2. Two rabbits, A and B, from the same litter, urethane, blood pressure in each from the carotid, both vagi in each exposed. Rabbits placed side by side. On the abdomen of each an electrode of the porous pot type was placed, and the lint was soaked in each case in a 1 per cent solution of atropine sulphate.

The size of electrode (20 sq. cm.) and all other details of arrangement were made precisely the same in the two animals, the porous pot elec-
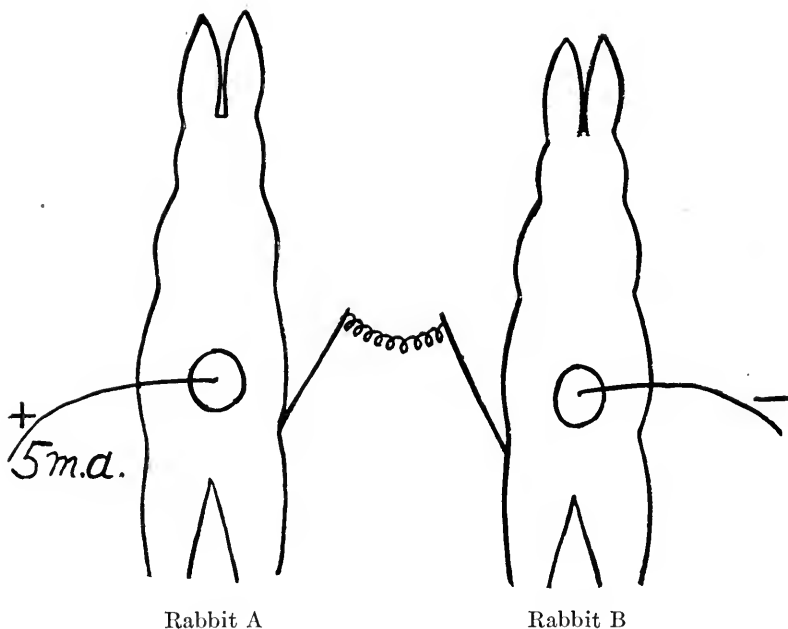

Rabbit A   Rabbit B

Fig. 1

trode on rabbit A was connected with the positive terminal. The other electrode of this rabbit consisted of a needle inserted in the thigh. This was connected with a similar needle in the thigh of rabbit B. The porous pot electrode on B was connected with the negative terminal of the source of current, and a current of 5 m.a. was now passed through the two rabbits thus arranged in series (see fig. 1). In eight minutes the vagi in rabbit A had almost ceased to respond to electrical stimulation. The vagi in rabbit B were unaffected (see fig. 2).

From these two experiments it is clear that under the conditions described atropine is absorbed into the general circulation producing paralysis of the cranial autonomic nerves.

In the rabbit it is not easy to produce complete paralysis, since this animal possesses considerable tolerance to this alkaloid owing to the rapid excretion and partial destruction of the atropine.   In experiment 2 it appears that when the vagi are almost paralyzed the rate of excretion and destruction keeps pace with the rate of absorption.

Perhaps the most remarkable feature connected with this form of absorption is the very small amount of current often necessary to produce an effect.   Thus if a strip of sheet zinc 1 cm. wide and

Rabbit A   Rabbit B

R   L   R   L
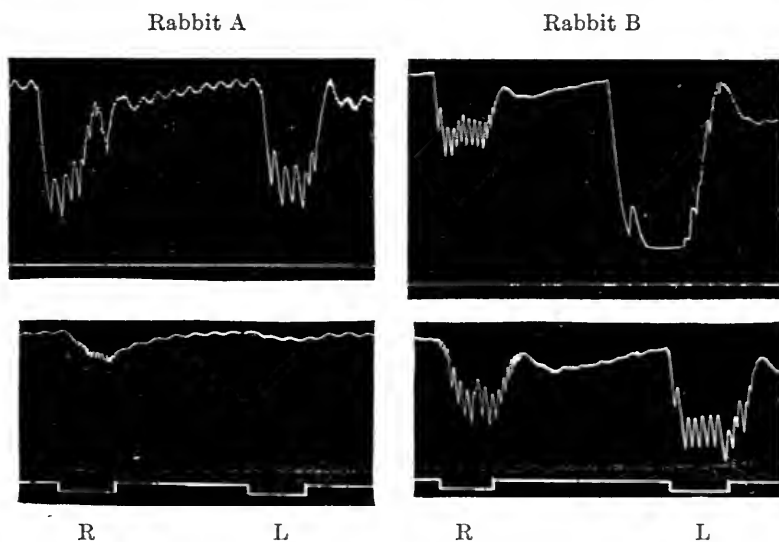
FIG. 2.   Blood pressure in two lower tracings lowered 20 mm.

10 cm. long be soldered to a sixpence it may readily be bent into a U, one arm being zinc and the other silver (fig. 3); then one end of each arm may be applied to either side of the tongue. If now pieces of moistened lint be placed between the silver and the tongue and between the zinc and the tongue a current of about 0.4 m.a. passes through the tongue from zinc to silver. Should now such a zinc-silver couple be applied to the anæsthe-tized cat's tongue in which the lint between the tongue and the zinc has been soaked in a 1 per cent solution of atropine sulphate complete paralysis of the cranial autonomic nerves occurs in from five to ten minutes.   Should however the atropine be applied to the other pole, between tongue and silver, no effect could be

observed in one hour although the atropine had been here in
contact with a mucous membrane.

Strychnine. Strychnine is absorbed like atropine provided the
conditions already described are the same. If a rabbit be anæsthe-
tized with urethane (1.5 grams per kilo body weight) then a 1
per cent solution of strychnine, placed on the positive pole
exactly as in experiment 1, causes a return of spinal reflexes in
five minutes and twitchings in about ten minutes. By increasing
the current true opisthotonus may be obtained. For this experi-
ment urethane is not the ideal anæsthetic, since it exerts a much
more decided effect on the spinal cord than ether or chloroform,
and is therefore a better antidote to strychnine.
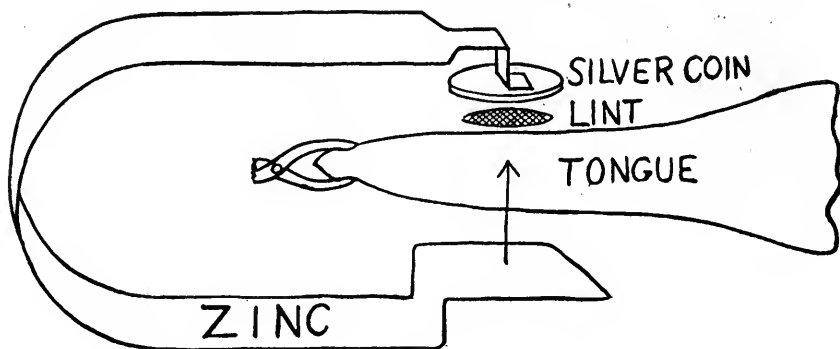
Fig. 3

Aconitine. Aconitine has such a characteristic action on the
heart in poisonous doses that its presence in the body is readily
detectable by physiological means.

Experiments were performed on rabbits anæsthetized with
urethane with a record of blood-pressure, using a 1 per cent
solution of aconitine hydrochloride on the positive electrode
which was placed on the abdomen. The electrodes were arranged
in every detail as in experiment 1. A current of 25 m.a. was
passed through an area of 20 sq. cm. The heart was clearly
affected in seventeen minutes, delirium cordis was decided in half
an hour and death occurred in an hour and a quarter (see figure 4).

Adrenaline. Two experiments were made using adrenaline

hydrochloride 1 : 1000 on the rabbit but they failed completely to prove any absorption into the general circulation, using as tests for the presence of adrenalin the blood-pressure and intestinal movements of the experimental animal.   This result is not surprising in view of the fact that the subcutaneous injection of two or three minims of the same strength of adrenaline failed to produce any action.
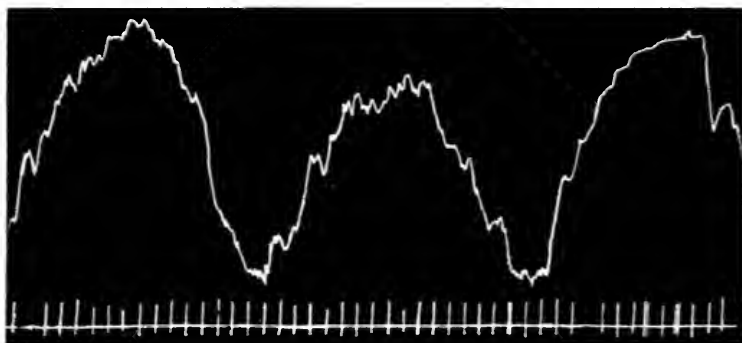

FIG. 4.   RABBIT, 2.5 gm.   URETHANE

One hour after ionizing with aconitine. Shows delirium cordis. Death one and one-quarter hours from beginning.   Blood pressure lowered 38 mm.

Metals

Calcium.   Calcium was one of the metals selected because it is possible to detect its presence in the blood by physiological means, namely the clotting-time of a drop of blood.

Experiment 3.   Rabbit, urethane.   Ear shaved the day previously. Drops of blood were obtained from the ear and tested for clotting-time under constant conditions by a method described elsewhere (3).   The current was conveyed through two porous pot electrodes connected one to each hind leg by strips of lint.   The lint on the positive pole was soaked with calcium chloride 1 per cent in distilled water; the lint on the negative pole with 1 per cent salt solution.

A current of 20 m.a. was now passed through approximately 40 sq. cm. of skin.

The following normal clotting times in seconds were obtained at

intervals of ten minutes: 100-, 100, 104.   After ionization with calcium
for 35 minutes clotting time was 70 seconds, after 40 minutes 66 sec-
onds.   After 65 minutes the current was stopped.   After 70 minutes
clotting time was 45 seconds and after 75 minutes 48 seconds.   Another,
experiment under similar conditions, but with a current of 25 m.a.
through a positive electrode on the abdomen with an area of 20 sq. cm.
gave similar figures.   Consecutive normal clotting times in seconds,
165, 183, 197, 209.   After ionization with calcium for 30 minutes
clotting time was 284 seconds, after 40 minutes 99 seconds, after 45
minutes 102 seconds.   After 65 minutes the current was stopped
After 70 minutes clotting time was 118 seconds, after 74 minutes 75
seconds, 78 afterminutes 79 seconds, and after 82 minutes 86 seconds.

This affords evidence that calcium is absorbed through the
skin under these conditions.   The observation seems of some
practical importance since it is not possible to increase the calcium
content of the blood of normal animals and man by the adminis-
tration of calcium salts by the mouth (4).

Iron.   Experiments were made with ferrous sulphate solution
and it was shown that after the application of the current for
half an hour the amount of iron was considerably increased in the
skin and subcutaneous tissues.   The control experiments in these
instances were made by applying the ferrous sulphate in a similar
way to another portion of the skin through which no current was
passing.

Experiment 4 (a) Rabbit, 2.5 kilos, urethane.   Hair clipped over
experimental areas.   The areas were in corresponding positions on
either side of the abdomen.   The control area had upon it a pad of
lint soaked in a solution of ferrous sulphate in distilled water, over
this and pressing the lint on the abdomen was a porous pot containing
salt solution.   The experimental area was arranged in an identical
fashion, except that a carbon rod, connected with the positive terminal
of the source of current, was placed in the salt solution.   The negative
electrode was a steel needle inserted into the hind leg of the same side.
The lint in each case covered the base of the pot and was 20 sq. cm. in
area.   A current of 30 m.a. was passed for one hour.

The animal was killed with chloroform.   Small portions of each skin
area with the adjacent muscles were excised, and thoroughly washed

in distilled water.   The skin was separated from the muscle and the four portions, two muscular and two skin, were placed in four weighed crucibles and dried in the incubator for twelve hours and subsequently in a desicator.   The weight of the dried tissues was determined, after which they were incinerated to ash.   The ash was dissolved in dilute. sulphuric acid by the aid of heat, and the solution was transferred to a glass vessel where the iron was reduced by metallic zinc in an atmosphere of carbon dioxide.   The iron was estimated by standard permanganate. The following figures show the percentage of iron to the dry weight of tissue.

	Experimental	Control
Skin	0.338	0.055
Muscle	0.186	0.056

Experiment. 4 (b) A further experiment confirmed the increase in iron in the skin and subcutaneous tissue, the percentage of iron to weight of ash after incineration is given.

In this experiment a current of 25 m.a. was passed for thirty minutes.

	Experimental	Control
Skin	43	13
Muscle	6	..

Experiment. 4 (c) In this experiment the rabbit was treated as in experiment 4 (a).   A current of 30 m.a. passed for one hour.   The tissues were handed over to Dr. J. F. Gaskell for histological examination by means of the Prussian blue reaction.   Sections were made through the whole thickness of the abdominal wall including skin and peritoneum, the tissue having been fixed in formol-Muller solution, imbedded in gelatine, and cut frozen.

The following is Dr. Gaskell's report: Sections of the electrically treated tissue gave a very deep iron reaction throughout the skin proper.   The supporting tissue of the subcutaneous fat was also a deep blue.   The reaction was especially intense round the vessels of the skin and also on the surface where hairs were emerging.   The color chiefly lay in the fibrous tissue and its special concentration in this tissue around the vessels pointed to an absorption by the lymphatic system. The cells forming the hair follicles were completely unstained.   The epidermis was deeply stained.   The deepest layer of fibrous tissue which showed stain was that just below the panniculus carnosus which forms a thin muscle sheet in the deepest layer of the skin.   The staining here was not intense but the supporting tissue of the muscular fibers themselves was fairly deeply stained.

All tissues lying deeper, both the muscle layers of the abdominal wall, their supporting tissues, and the peritoneum showed no sign of coloration. The staining was thus confined to the skin layers. Sections of the control tissues also showed a similar distribution of color, but its intensity was very much less. The points of concentration were the same. The coloring of the individual fibers of the fibrous tissues, and the marked concentration of the iron round the perivascular lymphatics was very conspicuous owing to the general less intense coloration. The fibrous tissues of the deepest layers of the skin, including that supporting the panniculus carnosus, showed an intensity of color only a little less than that of the ionized skin. The deep muscular layers and peritoneum were again unstained. The histological evidence therefore points to an increased intensity of absorption owing to the electrical current, this absorption taking place along the same channels as in the control.

The depth of penetration is the same in both cases, but the increased intensity of color in the electrically treated tissue is mainly superficial to the chief vessels of the skin, indicating that the action of the current is to increase the rapidity of absorption of the iron along identically the same channels as in the control, namely, as directly as possible from the skin to the perivascular lymphatics which drain the skin layers.

Negative ions

The negative ions which were employed because of their ease in detection were cyanides, ferricyanides, and salicylates.

Cyanides. The cyanides exert a characteristic stimulation of the medulla which is detectable by a record of the respiration. Using a moderate current and placing potassium cyanide upon the negative electrode, some stimulation of respiration is clear in a few minutes, and death occurs in from twenty minutes to one hour according to the amount of current passed. The details are shown in experiment 5.

Experiment 5. Guinea-pig, urethane. Record of respiration by tambour on thorax. A current of 1 m.a. was used over an area of 1 sq. cm.

The negative electrode consisted of a block of carbon placed on 12 layers of filter paper wetted with 1 per cent potassium cyanide.

Beneath this a sheet of oil-silk, in which a window of 1 cm. square was cut, was placed upon the abdomen.

The positive electrode consisted of a steel needle placed in a thigh muscle.

A current of 1 m.a. produced increased depth of respiration in a few minutes and respiration was paralysed in about twenty minutes.

From this it is clear that the cyanide ion is absorbed from the negative electrode with comparative ease, that it gets into the general circulation and exerts its specific action on the central nervous system.

In other experiments, using a current of 1 m.a. over 1 sq. cm., respiratory failure occurred in forty minutes (fig. 5) in another

Normal   6½ min.   13 min.   23 min.   40 min.   41 min.
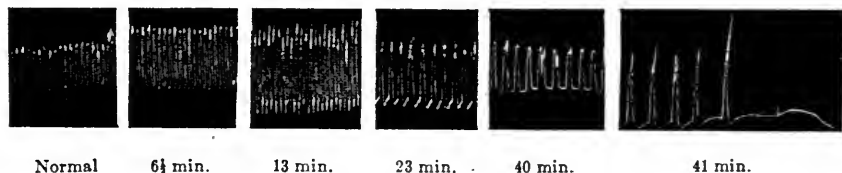
FIG. 5. GUINEA-PIG, 300 GRAM. URETHANE

KCN 5 per cent solution on negative electrode, 1 m.a. through area of 1 sq. cm. Record of respiration. Time 1 cm. = 16 seconds. Shows stimulation of respiration, and death in forty-one minutes.

using a current of 20 m.a. over an area of 10 sq. cm. the respiration was affected almost immediately and death occurred in seventeen minutes.

Salicylates. Salicylates can also be forced through the skin by this method, though perhaps not to the extent that enthusiastic writers on the subject would lead one to suppose.

In anæsthetized cats the urine was collected every ten minutes and the amount of salicylate determined. A current of 25 m.a. was passed for half an hour through a negative electrode of 20 sq. cm. on which sodium salicylate solution $\frac{N}{10}$ was placed.

In the first twenty minutes no salicylate could be detected in the urine. During the succeeding ten minutes 0.3 mgm. were found, and during the succeeding hour 0.5 mgm. Thus altogether less than a milligramme was found.

Salicylates are often employed in joint troubles, and experiments were therefore performed, using the knee-joints of anæsthetized rabbits.

The joint treated electrically was enveloped in an oil-silk or thin rubber sheet out of which a window of known area was cut over the joint. Over this was placed a pad of glass wool or lint soaked in sodium salicylate solution $\frac{N}{10}$. This was connected to the negative terminal of the source of current either directly by a tin-foil covering, or through the porous pot arrangement previously described. The positive connection was made by a steel needle inserted under the skin of the trunk.

The control joint was subjected to small subcutaneous injections of the same salicylate solution under the skin covering the region of the joint.

After the application of the current for half an hour the animal was killed, the skin removed from the joints, which were excised and opened for examination under a dilute solution of ferric chloride for evidence of salicylate in the parts.

In one experiment the subcutaneous injection about the region of the control joint amounted to only 0.5 cc. of salicylate solution, some slight difficulty being found in passing between the skin and the underlying tissues without puncturing them. The current employed was 30 m.a. through a window area of 2 by 1 cm. On final examination there was little, if any difference between the control joint and that treated electrically. In both cases the superficial tissues were deeply stained, together with the muscles, part of the articular cartilage of the femur and adjacent tendons. The semilunar cartilages and all deeper structures of the joint were unstained.

In another experiment, a subcutaneous injection of 1 cc. was made on either side of the control joint. A current of 15 m.a. was passed through a window-area of 3 by 5 cm.

In this experiment distinctly more staining was observed about the electrically treated joint: in it the superficial fascia and capsular ligament, the patellar tendon with its attachment to the tibia were intensely stained in the region underlying the window. The superficial aspect of the patella was intensely stained,

and some staining was present on the epicondular part of the femur where the ligaments were attached. On the other hand there was no staining within the knee-joint. The cruciform ligaments and the cartilages of the femur, tibia and patella were quite unstained. In the control joint there was no staining except of a small area of fascia and muscle at the outer side of the knee. The interior of the joint showed no staining. Thus under these conditions there was no evidence of deep penetration of the joint by salicylate.

In the next experiment the knee-joint of an anæsthetized cat was treated with sodium salicylate in the way described, and 5 m.a. was passed for half an hour through a window of about 5 sq. cm. The animal was killed and the skin covering the joint was carefully removed, avoiding contamination of its deep surface with salicylate, and the deep surface of the skin was compared with the immediately underlying fascia. On examination with ferric chloride solution the deep surface of the skin was intensely stained purple, although the adjacent fascia and the tissues of the joint itself showed only the slightest indication of staining. Thus there was no evidence that a current of this magnitude enabled the salicylate ion to penetrate deeper.

While discussing salicylates, reference may be made to experiments on the isolated intestine of the rabbit. Here a loop of about 6 inches of duodenum and jejunum was thoroughly washed out with water. A heavy nickel wire was inserted in one end which was then plugged, the wire extending about half the length of the loop; the wire outside the gut was suitably insulated by a covering of rubber tubing. The lumen of the gut was about half filled with decinormal sodium salicylate solution, and the other end of the gut was then plugged.

The loop was now placed in Tyrode's solution, oxygenated and at body temperature. It showed good peristaltic movements. In half an hour the Tyrode was changed. The nickel wire was connected with the negative terminal of a source of current and a wetted gauze strip was placed with one end in the Tyrode, the other connected metallically with the positive terminal, and a current of 3 m.a. was passed for half an hour.

The first sample of Tyrode contained 3.4 mgm. of salicylate. The second sample of Tyrode contained 29.9 mgm.

The experiment was repeated with the current reversed, the nickel wire in the gut being now connected with the positive terminal, the other conditions being identical.

The Tyrode after half an hour was found to contain only 3.8 mgm. of salicylate. .

Ferricyanides. Experiments, similar to those with the salicylate ion, were made with the ferricyanide ion upon the knee-joint of the rabbit.

An oil-silk window, 2 by 1 cm. in area, was placed across the front of the experimental joint, over this was placed a thick pad of lint soaked with potassium ferricyanide solution $\frac{N}{20}$. Above the pad was a layer of tin-foil connected with the negative terminal. The positive wire was connected with a steel needle introduced under the skin of the thorax. After a current of 10 m.a. had passed for a definite time, the animal was killed. The skin was removed from the knee-joints, and the excised joints were tested for the presence of ferricyanide. The joints were immersed in 5 per cent ferrous sulphate solution, opened out, and the amount of staining of the tissues observed.

Experiment 6. Rabbit, urethane. Experimental knee as above. Control knee treated exactly the same, except that no electrical connection was made. The current was applied for half an hour, and the animal killed.

Both knee-joints were placed in ferrous sulphate solution for twelve hours, then opened up and examined.

Control knee: All parts unstained except very slight staining on the anterior surface of the patella and adjacent capsular ligament.

Experimental knee: Anterior surface of patella and adjacent capsular ligament intensely stained. Joint cartilage of femur and tibia stained in places. Cartilage of patella stained at sides. Bone of patella unstained. Crucial ligaments unstained.

Experiment 7. Rabbit, urethane. Arranged as in preceding experiment. Current applied for half an hour and the animal killed. The excised unopened joints were immersed in ferrous sulphate solution for five minutes and examined.

Control knee: Unstained.

Experimental knee: Stained.

The joints were then opened up and placed in the solution for a further five minutes.

Control knee: Unstained.

Experimental knee: Capsular ligament, joint cartilage of femur and internal semilunar cartilage stained in parts which were under the electrode.   Crucial ligaments unstained.

Experiment 8.   Rabbit, urethane.   Experimental knee arranged as before.   Control knee received a subcutaneous injection of 0.5 cc. of $\frac{N}{20}$ potassium ferricyanide solution over the joint.   The current was applied for a quarter of an hour and the animal killed.

The excised unopened joints were placed in the ferrous sulphate solution for five minutes and examined.

Control knee: Periarticular tissues stained.

Experimental knee: Periarticular tissues stained.   The joints were then opened up and replaced in the ferrous sulphate solution for twelve hours and examined.

Control knee: Intensely stained.   Periarticular tissues: joint cartilage of femur in one place; joint cartilage of tibia in one place. Crucial ligaments unstained.

Experimental knee: Stained.   Periarticular tissues; joint cartilage of femur in one place.   Intensely stained.   Patellar tendon and capsular ligament as far as the joint.

Unstained.   Joint cartilage of tibia; crucial ligaments.

The experiments on the knee-joint with salicylate and ferricyanide described above show clearly that absorption through the skin is facilitated by the electric current.   But the current caused no deeper penetration into the joint than could be obtained by simple subcutaneous injection of the drug under the skin covering the joint.

Thus with ferricyanide there was more intense staining of the deeper tissues (cartilages of femur, tibia) when injected subcutaneously than when introduced by the current.   With salicylate no penetration into the joint occurred either when introduced electrically or when subcutaneously injected.   With this drug, in one experiment, the superficial tissues (outer surface of capsular ligament, patellar tendon and fascia) were equally well stained by both methods of introduction, while in another experi-

ment there was intense but superficial staining in the electrically treated joint, with but very slight staining in the injected one.

Under the conditions described there is no evidence that the current has the power to convey these drugs deeply into the tissues beneath.

SUMMARY OF CONCLUSIONS

1. Both positive and negative ions can be made to be absorbed through the skin or mucous membrane by means of the electric current if solutions of the drugs are applied at the appropriate electrode.

2. The current is conveyed through the body by the tissue ions, after the introduction into the body of the foreign ion the current has no further appreciable influence upon it. With a current localized in the tongue the atropine ion rapidly reaches the heart. The ferricyanide ion penetrates through the skin but after this the electric current has no further influence.

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ON THE INFLUENCE OF COLLOIDS ON THE ACTION OF NON-COLLOIDAL DRUGS. III

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It had been shown in previous communications that the action of several drugs can be inhibited by the addition of colloids. Rabbit serum contains substances which are able to inhibit the action of pilocarpine and atropin (1, 2, 3); brain substances can inhibit the action of cocain (4) and of strychnin (Sano (5)) and so forth.

In former publications it was pointed out that this phenomenon (i.e., the inhibitory influence of colloids on drugs) possesses two interesting features:

1. The inhibitory action of colloids is a very specific one, that is to say some colloids may inhibit the action of a certain drug and other—closely related—colloids may not.

2. The inhibition is brought forth not by a chemical destruction of the drug, but by an adsorption of the drug or at least by a physical chemical process, closely related to adsorption processes.

In the course of the experiments mentioned above, it was noticed that colloids cannot only *inhibit* the action of alkaloids, but that they can also *increase* this action. For instance, rabbit serum may inhibit the action of pilocarpine on the isolated gut, but it may also—if the conditions of the experiment are altered—increase the pilocarpine action. To demonstrate the *inhibitory* effect of a rabbit serum one may proceed as follows:

To 0.9 cc. of rabbit serum (which has been kept in the ice-box for at least twenty-four hours) is added 0.1 cc. of a 10 per cent solution of pilocarpine hydrochloride and the mixture is kept—after agitating—at room temperature for fifteen to thirty minutes or longer. After that time the strength of the serum—pilocarpine

solution is determined by standardizing it on the isolated gut using a solution of pilocarpine in water as a standard. It will be found then, that about $\frac{18}{20}$ to $\frac{38}{40}$ of the pilocarpine from the serum-solution has disappeared or at least has been rendered into an inactive form.
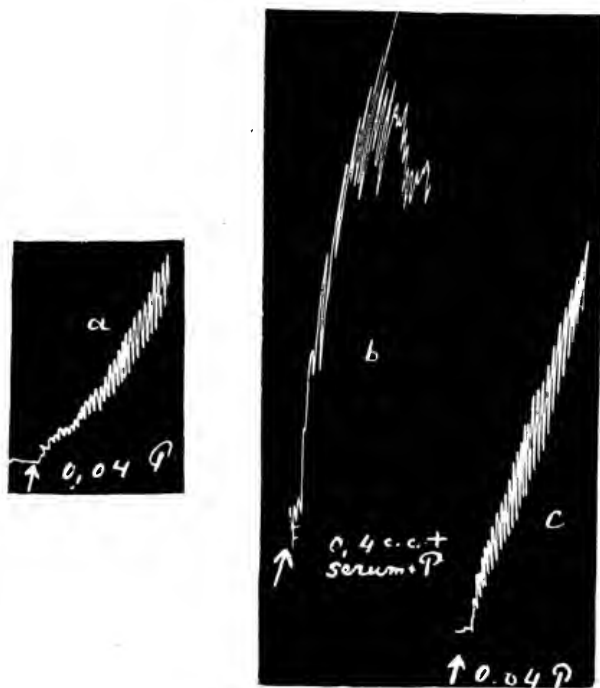
FIG. 1. ISOLATED GUT SUSPENDED IN 75 CC. OF TYRODE SOLUTION. IN-FLUENCE OF SERUM ON PILOCARPINE ACTION

a, Action of 0.04 mgm. of pilocarpine hydrochloride; *b*, pilocarpine plus serum is added to the gut, the quanity of pilocarpine and serum is immaterial; *c*, 0.04 mgm. of pilocarpine (same doses as in *a*) gives a much stronger contraction. In this figure and in all the following figures the influence of a colloid has never been tested before it had been shown, that reactions of the gut to a certain dosis of pilocarpine had become constant.

In performing a standardization experiment in the way described one often notices that after a small dose of serum has been added to the gut (and subsequently has been washed out again) the sensitiveness of the gut to a further dose of pilocarpine has increased. Figure 1, *a*, illustrates this very clearly.

In figure 1, *a*, 0.04 mgm. of pilocarpine hydrochloride is added to a piece of gut suspended in 75 cc. of Tyrode solution and after three minutes this pilocarpine is washed out; in figure 1, *b*, a certain amount of pilocarpine plus rabbit serum is added and is also washed out after three minutes; in figure 1, *c*, again 0.04 mgm. of pilocarpine is added (the same quantity as in figure 1, *a*) this dose now produces a much stronger contraction than in figure 1, *a*.

This increase of pilocarpine action after the addition of serum is often seen in experiments such as are described here; it is however not a constant phenomenon.   In making a preliminary study of the problem (6), we found the promoting influence of serum to appear 15 times in 20 experiments; the serum was inactive in one case and in 4 cases the serum seemed to lessen the pilocarpine action a little.

We made an attempt to identify the substances in rabbit serum which are responsible for the described effect and first made a study of some of the lipoids which are known to occur in serum.   Through the kindness of Dr. Levene of The Rockefeller Institute we were able to extend our studies to some lipoids derived from brain tissue.   In our first experiments in this direction we found that lecithin and cholesterin increase the action of pilocarpine on the isolated gut.   It must be added however, that the described action of lecithin and cholesterin is not a constant one.   In our first experiments when a sample of lecithin from Merck which had been kept in the laboratory for years, was used, we got fairly constant results; the lecithin nearly always increased the pilocarpine action.   Later however we examined a sample of lecithin (Levene) derived from brain tissue.   With this sample we obtained the following results.

The action of lecithin was investigated in 13 experiments. In 6 cases the lecithin was inactive, in 5 cases it had a distinctly promoting effect on the action of pilocarpine and in 1 case the action of pilocarpine seemed to be weakened a little by lecithin. Figure 2 gives an instance of a positive lecithin experiment.   In this experiment we first determined the sensitiveness of the gut for pilocarpine and after it had been established that 0.01 mgm. of

pilocarpine gave in three successive cases a slight contraction of the same intensity, one drop of 5 per cent lecithin emulsion was added, the pilocarpine effect was strongly increased. This figure shows also that this amount of lecithin did not per se stimulate the gut, because after washing out the effect of lecithin plus pilo-carpine, one drop of the 5 per cent lecithin emulsion alone had no influence, whereas an additional dose of pilocarpine then gave a strong contraction. Moreover in other cases we found that the lecithin action persisted after the lecithin had been washed away.
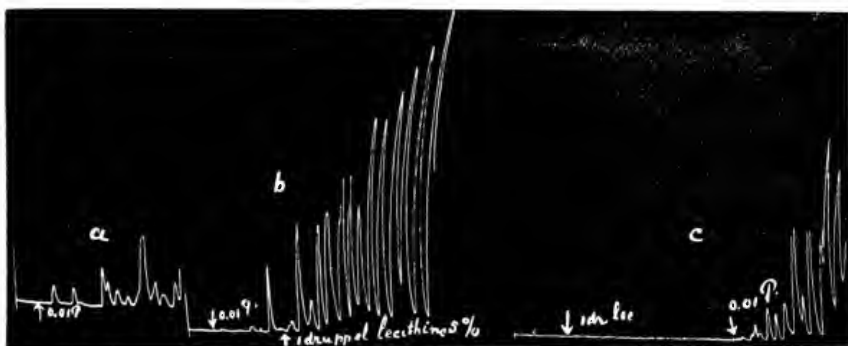
FIG. 2. INFLUENCE OF LECITHIN ON PILOCARPINE ACTION

a, Action of 0.01 mgm. of pilocarpine; *b*, first 0.01 mgm. of pilocarpine is given and then 1 drop of a 5 per cent emulsion of lecithin; *c*, first 1 drop of lecithin is given showing that this has per se no influence at all on the gut; after some minutes 0.01 mgm. of pilocarpine is added.

In this experiment then it is shown that lecithin can promote the action of pilocarpine on the isolated gut and can do so in doses which are per se unable to stimulate the gut. Moreover it was shown that lecithin has this action when it is given at the same time as the pilocarpine and also when first the lecithin is given and afterwards the pilocarpine; in the last case it is some-times possible to wash out the lecithin without reducing the positive effect on the pilocarpine action. We desire again to lay stress on the fact that lecithin does not always do so.

The next lipoid to be investigated was cholesterin. Here also we found a distinct effect, but here also the phenomenon is not

always demonstrable.   Figures 3 and 4 give instances of a distinct promoting effect of cholesterin.   In figure 3, *a*, a piece of isolated gut was suspended in 75 cc. of Tyrode solution; addition of 0.1 mgm. pilocarpine hydrochloride gave a moderate contraction and after the drug had been "washed out" a subsequent dose of the same strength gave exactly the same response.   We repeated
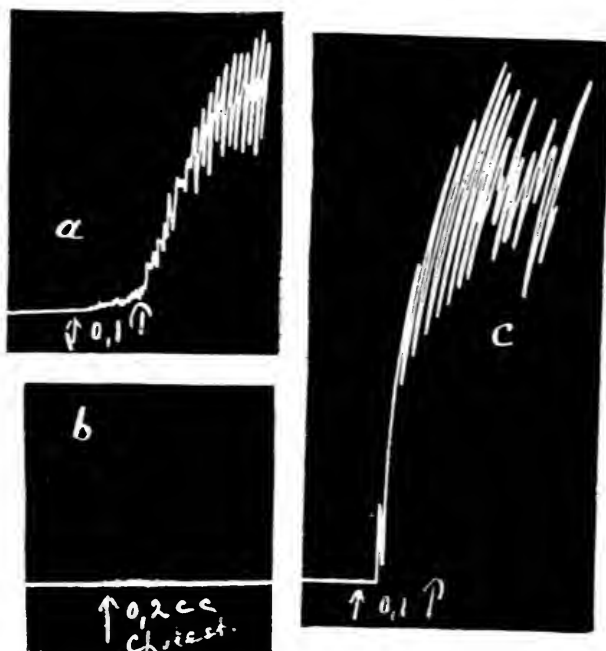
FIG. 3. INFLUENCE OF CHOLESTERINE ON PILOCARPINE ACTION

a, Action of 0.1 mgm. pilocarpine; *b*, 0.2 cc. of cholesterine emulsion has no influence; *c*, addition of 0.1 mgm. pilocarpine after cholesterine gives stronger contraction than 0.1 mgm. pilocarpine alone did before.

this three times and figure 3, *a*, gives the third reaction to 0.1 mgm.   After this dose of pilocarpine had been washed out we gave 0.2 cc. of a cholesterin emulsion (fig. 3, *b*).   This cholesterin was left in the vessel and 0.1 mgm. of pilocarpine was now added, fig. 3, *c*, which gave a much more powerful contraction than it did before the addition of cholesterin.   To rule out the possibility that cholesterin (which may in large doses stimulate smooth

muscles) had in the experiment under discussion only added its subliminal action to that of the pilocarpine, we performed the experiment represented in figure 4. Here 0.03 mgm. of pilocarpine gave a constant effect on a piece of isolated gut (fig. 4, a). We
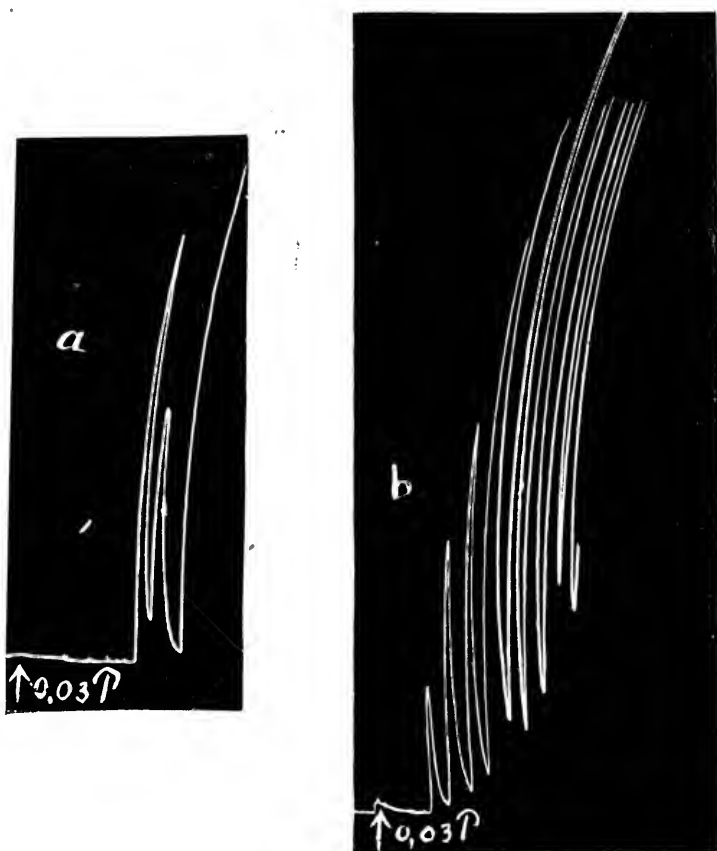
FIG. 4. INFLUENCE OF CHOLESTERINE ON PILOCARPINE ACTION

a, Action of 0.03 mgm. of pilocarpine; b, action of same dosis of pilocarpine. Between a and b the gut has been suspended in 150 cc. of Tyrode solution, to which 0.5 cc. of a 1 per cent cholesterine emulsion had been added.

now washed out the pilocarpine and added 0.5 cc. of a cholesterin emulsion to the Tyrode solution in which the gut was suspended and left this in contact with the gut for fifteen minutes. The cholesterin was then washed out and after a few minutes 0.03
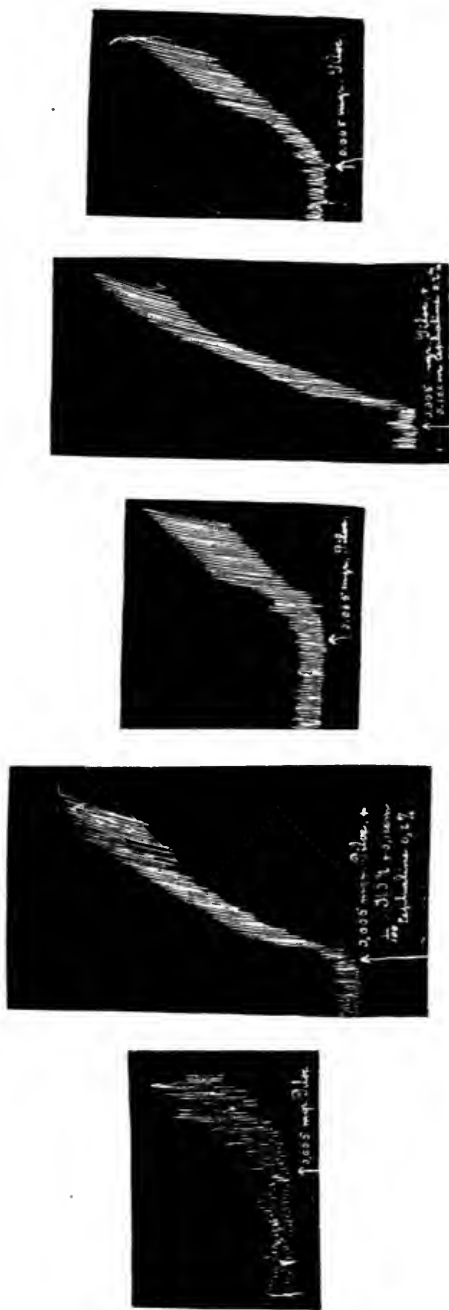
FIG. 5. INFLUENCE OF CEPHALIN ON PILOCARPINE ACTION

a, Action of 0.005 mgm. of pilocarpine; *b*, action of 0.005 mgm. of pilocarpine which has been in contact with 0.1 cc. of cephalin solution 0.2 per cent; *c*, action of 0.005 mgm. of pilocarpine; *c*, *d*, Action of 0.005 mgm. of pilocarpine plus cephalin; *e*, action of 0.005 mgm. of pilocarpine alone.

mgm. pilocarpine was added (fig. 4, *b*), which produced a much stronger contraction than the same dose had given before. This experiment proves that the promoting influence of cholesterin on pilocarpine can persist after the greater part of the substance had been "washed" away. We could repeat this experiment several times but—as in the case of lecithin—this cholesterin action is not always present; for an unknown reason the cholesterin may fail to act.

After this interesting effect of lecithin and cholesterin had been found we studied other lipoids, i.e., cerebron and cephalin. As we were in possession of only a small quantity of cerebron, we only performed three experiments with this lipoid. In all these cases it had a distinct, though slight effect on the pilocarpine action. The action of cephalin was much more pronounced, but we here met with the same difficulty as in the case of lecithin; the cephalin action was not constant though it gave better results than lecithin. Figure 5 gives an instance of a very clear case of the increasing effect of cephalin on the pilocarpine action.

In figure 5, *a*, 0.005 mgm. of pilocarpine hydrochloride were added to the gut and this dose gave a moderate contraction. This curve is the last one of a series of three successive curves of exactly the same shape; the dose of pilocarpine added being the same in all three cases, so that it could be taken for granted that the sensitiveness of the gut had become constant. We took this same precaution in all the experiments described in this paper, the influence of a colloid never being tested until it had been proved by the three of four successive additions of pilocarpine that the sensitiveness of the gut to this drug—which may in the beginning of an experiment vary considerably—had become constant. Moreover we always after each addition of a foreign drug or a colloid made a control with the fixed quantity of pilocarpine to investigate whether the sensitiveness of the gut had altered.

In figure 5, *b*, 0.005 mgm. pilocarpine plus 0.1 cc. of a 0.2 per cent cephalin suspension was given to the gut and the pilocarpine action is much stronger than in figure 5, *a*. In figure 5, *c*, again 0.005 mgm. of pilocarpine was given and the resulting contraction

was smaller than in figure 5, *b,* but stronger than in figure 5, *a,* which may be taken as an indication that the cephalin has some "after effect." In figure 5, *d,* again pilocarpine plus cephalin was given and in figure 5, *e,* pilocarpine alone.

It must be remarked here, that the action of cephalin as well cannot always be demonstrated. We tried to obtain more information on the conditions which determine whether the cephalin will or will not act as a promotor of pilocarpine action; until the present time however we have not obtained definite results. Cephalin is practically insoluble in water, so that in order to study the influence of this substance we had to try to obtain a fine aqueous suspension. This was not always easily done. We got the impression that the degree of dispersity plays an important part in this matter. Experiments are now in progress in this institute to investigate the question with the help of modern physico-chemical methods in a more accurate manner.

As our researches with cephalin were badly handicapped by the fact that we had only at our disposition a limited quantity of this material we tried to find another substance which would show the same peculiarities in action as the cephalin but which would be more easy to obtain. We therefore studied the influence of several substances on the action of pilocarpine with the following results.

Protargol (collargol) showed a distinct promoting influence in three cases, but was inactive in other instances.

Nucleic acid was also slightly active sometimes, but not constantly.

Starch, bolus alba, gelatine, agar agar, kaoline, stearinic acid, salts, caseine, hæmoglobine, egg-white were all negative.

Pepton had in a number of experiments a very distinct promoting effect on the pilocarpine action. As it can exert, however— also an inhibiting effect on the pilocarpine action so that it could not further be used in this study.

As will be seen from this list of substances we did not succeed in getting a good substitute for cephalin. Some substances, as collargol and nucleic acid, may show a very definite promoting

influence upon the pilocarpine action, but their action nevertheless is so inconstant that they cannot serve our purposes.

The two substances which gave the best results and which were easily obtained were lecithin and cholesterin and therefore we determined to make a further study of the behaviour of these substances, being aware of the difficulties to be met on account of their inconstant action.

We first wanted to know whether this peculiar intensifying action of lecithin was limited to an influence on pilocarpine or whether the action of other drugs also might be promoted by lecithin. One of the most interesting drugs to be studied in this respect seemed to us to be histamine, this poison being present not only in the blood, but also ⸱ı all animal tissues, for we thought that the promoting action of colloids on this poison could also explain some very interesting facts, recently observed by Novy, Bordet and others. These authors stated namely, that fresh guinea-pig serum can be rendered highly poisonous, simply by the addition of some indifferent colloids (as agar agar). The picture of the poisoning was very similar to that seen in anaphylactic shock. If a promoting action of colloids on histamine could be demonstrated, this artificial anaphylaxis could possibly be explained in a simple way. It might be possible that such a promoting action of the colloid on some histamine like poison, present in normal blood, might account for these anaphylactic symptoms. Our supposition of a promoting action of colloids on histamine was tested and found to be correct. The following curves will demonstrate the increasing effect of cholesterin and lecithin on the action of histamine.

In figure 6, a, 0.06 mgm. of histamine (Ergamine Burrough, Welcome & Company) gives a slight contraction, after this dosis of histamine has been washed out a small quantity of a 1 per cent lecithin emulsion is added to the gut (fig. 6, b), which gives a slight reaction. After five minutes 0.04 mgm. of histamine is added (viz., a smaller dose as given in fig. 6, a) and a strong contraction results. After having washed out both the histamine and the lecithin, in figure 6, c, again 0.06 mgm. histamine is given, which produces a contraction of the same strength as in figure 6, a.

In figure 7, *a*, 0.01 mgm. of histamine produced a moderate contraction; the same dose of histamine which had been in contact with 1 cc. 1 per cent lecithin gave a stronger contraction in figure 7, *b*, whereas in figure 7, *c*, 0.01 mgm. alone gave a weaker effect than in figure 7, *b*, but a stronger effect than in figure 7, *a*, showing that very likely the lecithin had an after effect here.

The promoting effect of a colloid on the action of a certain drug, logically can be explained in two ways. Firstly it can be assumed that the colloid renders the gut more sensitive to the drug action. The second way of explanation is to suppose, that through some reaction between colloid and drug the latter is rendered more effective, in a manner analogous to the case of cobra-poison and lecithin.
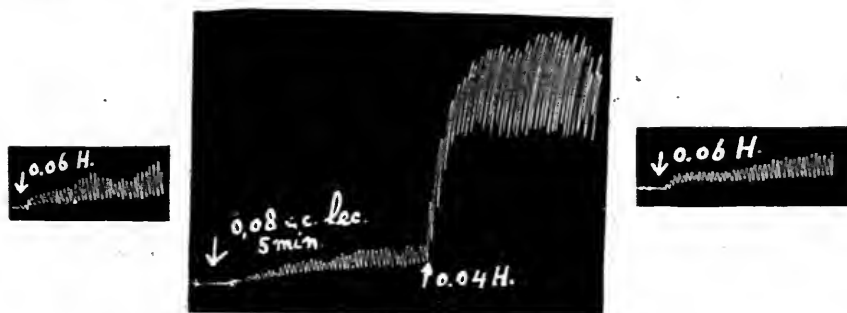
FIG. 6. INFLUENCE OF LECITHIN ON HISTAMINE ACTION

a, Action of 0.06 mgm. of histamine (ergamine B. W & Co.); *b*, addition of 0.08 cc. of 0.3 per cent lecithin emulsion and after five minutes 0.04 mgm. of histamine; *c*, action of 0.06 mgm. of histamine.

This question could be settled by means of the following experiments. If the lecithin acts by making the gut more sensitive, it must exert its strongest action, if allowed to be in contact with the gut, before histamine has been added (i.e., if we first add lecithin, and some time later histamine to the gut). If the lecithin acts by making the drug more active, it must be most effective, if allowed to be in contact with the drug before being added to the gut (i.e., if we give the lecithin in a mixture with histamine, which has been made up for some time). In this second case a quite fresh mixture of histamine with lecithin must be much less

effective than an old mixture, whilst in the first case, in which lecithin is supposed to have an action on the gut, both mixtures, the fresh and the old one must equally be effective.

. In this way the two possibilities mentioned above were tested, but on this point as well we could not obtain quite constant

FIG. 7. INFLUENCE OF LECITHIN ON HISTAMINE ACTION

a, Action of 0.01 mgm. of histamine; *b*, 0.01 mgm. of histamine which has been in contact for some time with 0.1 cc. of 1 per cent lecithin emulsion; *c*, 0.01 mgm. of histamine.

reactions. In some cases the experiments showed the lecithin to act so as to render the gut more sensitive, in the other cases the lecithin seemed to have an action on the histamine, whilst in the other cases the lecithin seemed to be entirely ineffective.

We cannot yet give any deeper analysis of this phenomenon, and as a preliminary we only wish to state, that very likely colloids can promote drug-action in a double way, both by

rendering an organ moresensitive and by rendering a drug more effective. This problem is being studied in this institute at the present moment. The results will be published later. We wish however to call attention to the fact, that in our recent studies we found that the action of drugs cannot only be promoted by the colloids mentioned in this paper, but in some case also by ultra-filtrates or by dialysates of these colloids. This fact is in agreement with a statement made by one of us together with Miss v. d. Made (7) i.e., that dialysate of pepton can under certain circumstances increase the action of adrenalin on blood-pressure. It must then be considered highly probable that part of the promoting effects of colloids described in this paper are to be ascribed to the action of substances, which pass through ultra-filters or dialysating membranes.

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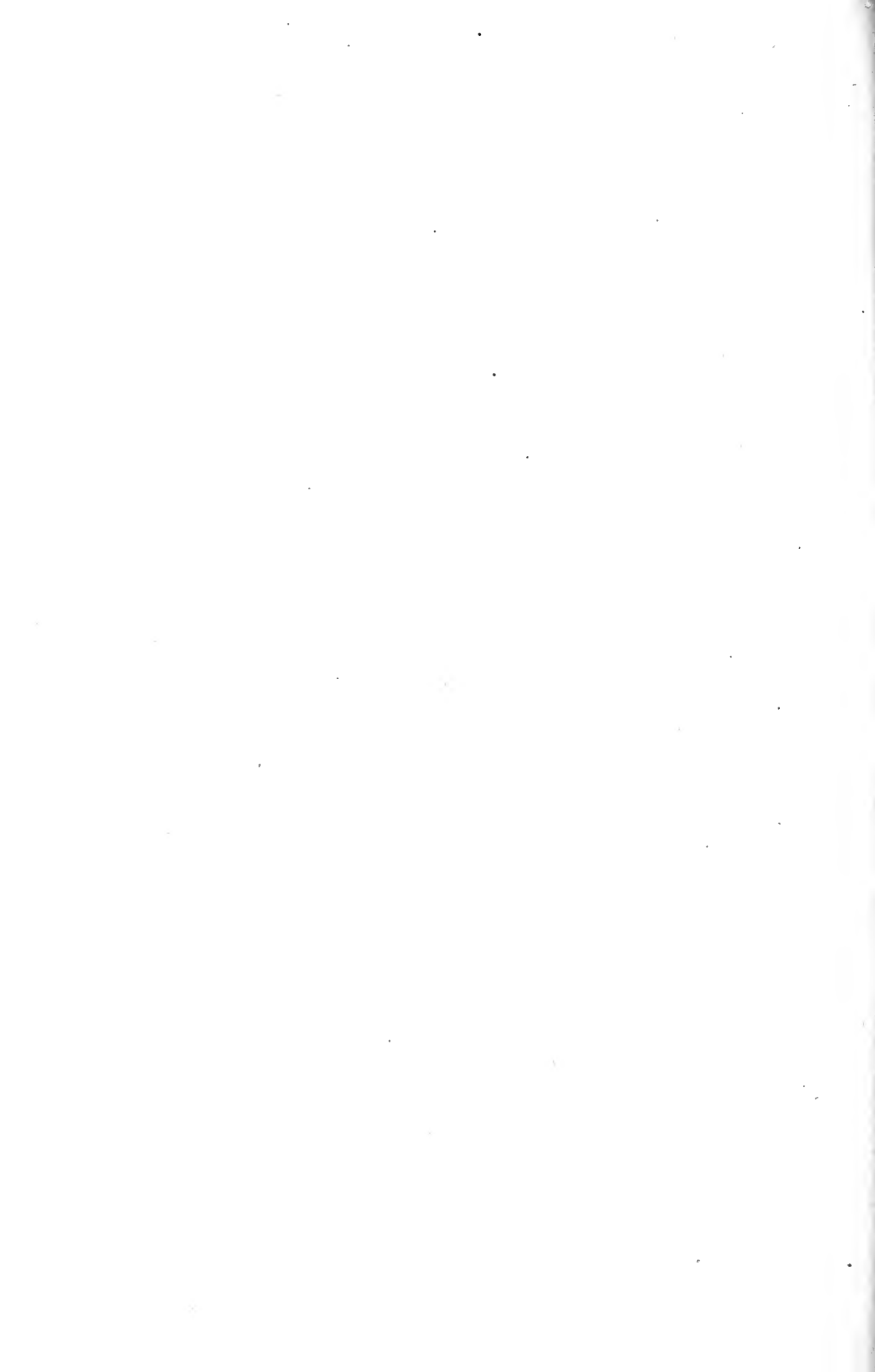
ON THE INFLUENCE OF COLLOIDS ON THE ACTION
OF NON-COLLOIDAL DRUGS. IV

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In the previous communications of this series (1, 2) it was shown that rabbit serum contains substances which have the property of inhibiting the action of atropin and pilocarpine by means of a physico-chemical process, presumably an absorption. Our first experiments on the inhibition of the action of pilocarpine by colloids were made in the Pharmacological Institute in Utrecht and, as described in the publication mentioned above, we found as a rule a very considerable binding action of rabbit serum. After moving to Leiden we continued this line of research with the intention of investigating whether, by repeatedly injecting small doses of pilocarpine into rabbits, the binding capacity of their serum could be increased. We were very surprised to find that in Leiden the rabbit serum had only a very slight binding power for pilocarpine. In Utrecht, when we added to 1 cc. of rabbit serum 10 mgm. of pilocarpine hydrochloride, we found, as a rule, that after standing at room temperature for ten minutes to one hour, 1 cc. of serum containing pilocarpine showed only the action of 0.5 to 0.2 mgm. of pilocarpine, so that 9.5 to 9.8 mgm. (i.e., 19/20 to 49/50 of the pilocarpine) had been made inactive.

In Leiden however we often found in similar experiments that from 10 mgm. of pilocarpine only 5 to 7.5 mgm. (i.e., $\frac{1}{2}$ to $\frac{1}{4}$ of the pilocarpine added) had been absorbed.

In looking for an explanation of this fact, we of course thought first of the possibility that the rabbits in Utrecht were of a different breed or perhaps were differently fed, and though we have found, as will be related presently, one other reason for the

271

different behavior of the animals in the Utrecht and the Leiden experiments, we cannot exclude the possibility, that differences in breed or in food also play some part.

In order to find out whether some alteration in the technique used could be responsible for the difference in the results of the earlier and of the later experiments we carefully reviewed our protocols and were only able to trace one difference between the experiments of the first and of the second series. In Utrecht we had as a rule taken blood from rabbits that had been killed without preceding narcosis, whereas in Leiden we wanted to make repeated examinations of the blood of the same animal and therefore we narcotized the rabbits before taking a sample of their blood.

Since we are of the opinion (as discussed in the third communication) that the phenomenon of the formation of anaphylatoxin in vitro may be explained in certain cases by the supposition that changes in the colloidal state of the serum can exert an intensifying influence on the action of toxic substances, already present in the serum in a less active form, and since it is known, that the formation of anaphylatoxin in vitro can be inhibited by the addition of ether we deemed it possible that the differences between the results of the Utrecht and the Leiden experiments could be explained by the fact that we had in the earlier cases used blood of normal rabbits and in the later cases blood of narcotized rabbits.

To test this possibility the following experiments were carried out.

Samples of blood were taken from rabbits without narcosis; the animals were then narcotized and bled again and finally the pilocarpine absorbing power of the different samples was determined with the method described in the first communication. As will be seen in the protocols given below the absorptive power of the rabbit serum can be greatly reduced by narcosis.

Experiment I

September 16, 1920. Rabbit. Sample (a) of blood taken from ear vein; the animal then narcotized for one-half hour with ether and again

bled (b). Both samples put in ice chest till next day. (This is necessary as fresh rabbit serum contains substances which *per se* stimulate the isolated gut).

September 17, 1920. Both samples examined for binding capacity on isolated gut.

a. Non-narcotized. To 1 cc. of serum is added 1 mgm. of pilocarpine hydrochloride. The mixture is allowed to stand for twenty-five minutes. The action of 1 mgm. of pilocarpine is reduced to nearly $\frac{1}{20}$.

b. Narcotized. Treated as a. Action of pilocarpine reduced to nearly $\frac{1}{5}$.

In this experiment then it was shown that the serum of a narcotized rabbit has less binding capacity for pilocarpine than the serum of the same rabbit before narcosis.

For shortness sake we propose to speak in cases like those mentioned above of the pilocarpine binding power of the serum being 20 and 5.

The pilocarpine binding power of a certain serum then has a value a if by the addition of 1 cc. of serum to 1 mgm. of pilocarpine hydrochloride the action of the pilocarpine on the isolated intestine is reduced to the 1/a part of its former strength.

It goes without saying that the proposed expression can only be used for cases in which to 1 cc. of serum is added 1 mgm. of pilocarpine hydrochloride. *If different quantities of pilocarpine or of serum are used the value for the binding capacity will vary without it being possible to make a calculation as to the degree of this variation.*

To corroborate the findings from experiment I we performed experiment II in the same way as experiment I.

Experiment II

Sample a. Non-narcotized. The action of 1 mgm. of pilocarpine is reduced to 1/17.5 accordingly the pilocarpine binding power was 17.5.

Sample b. Narcotized. Pilocarpine binding power 2.75.

Some phases of this experiment are represented in figure 1 a—f.

In figure 1 a and 1 c 0.05 mgm. of pilocarpine hydrochloride acts on the isolated gut. In figure 1 b the action of 0.15 mgm. of

pilocarpine plus serum of a narcotized rabbit is seen. This produces a stronger contraction than 0.05 pilocarpine did before, showing that the narcotized serum had a binding power less than 3.
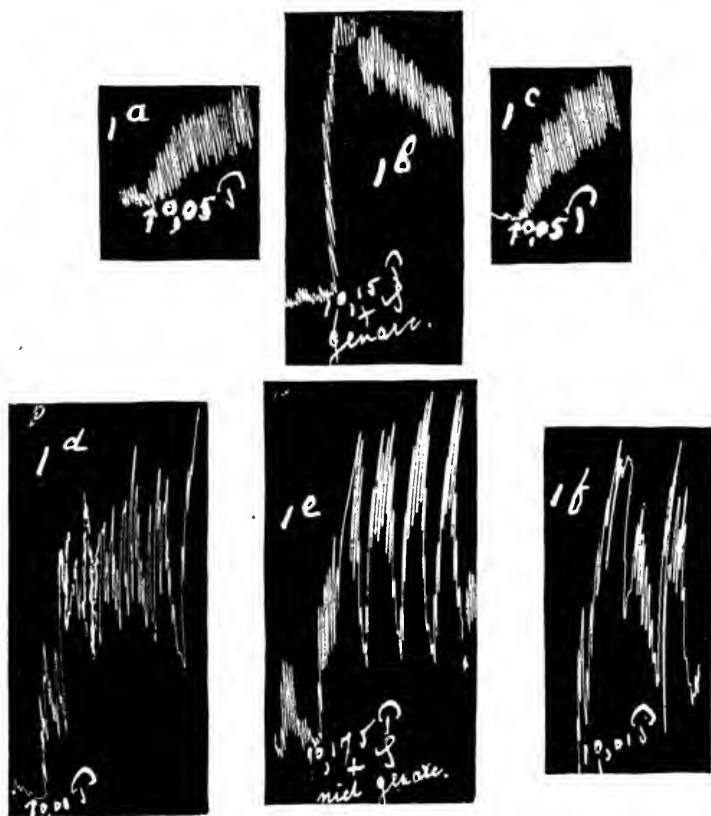

FIG. 1. ISOLATED CAT'S INTESTINE SUSPENDED IN 75 C.C. OF TYRODE SOLUTION

a and *c*, action of 0.05 mgm. pilocarpine hydrochloride, *b*, action of 0.15 mgm. pilocarpine plus serum of narcotized rabbit; *d* and *f*, action of 0.01 mgm. pilocarpine on another piece of cat's intestine; *e*, action of 0.175 mgm. pilocarpine plus serum of non-narcotized rabbit.

In figure 1 d and 1 f 0.01 mgm. of pilocarpine acts on another piece of isolated gut. In figure 1 e 0.175 mgm. of pilocarpine plus non-narcotized serum is given, this produces a contraction, nearly equal to that from 0.01 mgm. of pilocarpine, so that it may be concluded that the pilocarpine binding power is 17.5.

It was clear from these two experiments that serum from a narcotized rabbit had less capacity for binding pilocarpine than serum from a normal rabbit.

After having found the influence of ether on the binding capacity of the serum, the next step was to investigate whether the ether would exert the same influence in vitro. The following protocols show that this proved to be the case.

Experiment III

October 20, 1920. a. To rabbit serum—taken from a non-narcotized rabbit the day before—is added pilocarpine, so that 1 cc. serum contains 1.5 mgm. pilocarpine. The strength of this mixture is examined on the isolated gut. 0.4 mgm. of pilocarpine plus serum has a weaker action than 0.02 mgm. pilocarpine alone; 0.8 mgm. of pilocarpine plus serum has a stronger action than 0.02 mgm. pilocarpine alone. So that the pilocarpine binding power of the serum is more than 20.

b. Same experiment as in *a*, except that to the rabbit serum is added about 0,1 per cent ether. 0.02 mgm. of pilocarpine plus serum plus ether has a weaker action than 0.02 mgm. pilocarpine alone; 0.1 mgm. of pilocarpine plus serum plus ether has the sane action as 0.02 mgm. pilocarpine alone; 0.2 mgm. of pilocarpine plus serum plus ether has a stronger action than 0.02 mgm. pilocarpine alone. So that the pilocarpine binding power is 5.

Experiment IV

November 30, 1920. Same experiment as III. 0.01 mgm. pilocarpine plus serum has a weaker action than 0.005 mgm. pilocarpine alone; 0.01 mgm. pilocarpine plus serum plus ether has a stronger action than 0.005 mgm. pilocarpine alone.

In this experiment it was not possible to determine the binding capacity of the serum exactly, but it was clearly shown that this capacity was reduced by addition of ether.

Experiment V

October 1, 1920. Same experiment as III. 0.004 mgm. pilocarpine plus serum has a weaker action than 0.002 mgm. pilocarpine alone; 0.006 mgm. pilocarpine plus serum has a stronger action than 0.002

mgm. pilocarpine alone; the pilocarpine binding power of the serum is about 2. 0.003 mgm. pilocarpine plus serum plus ether has a weaker action than 0.003 mgm. pilocarpine alone; 0.004 mgm. pilocarpine plus serum plus ether has a stronger action than 0.003 mgm. pilocarpine alone.   The serum plus ether has hardly any influence.

Here also was shown that ether reduces the pilocarpine binding power of the serum.

Experiment VI

October 1, 1920. The rabbit serum used in this experiment had only a slight binding power for pilocarpine, this was however completely abolished by the addition of ether.

Experiment VII

October 7, 1920. This experiment was performed to determine whether the influence of ether on the pilocarpine binding power of the serum could be abolished by evaporating the ether from the serum. We therefore left the serum plus ether at 37° during $1\frac{1}{2}$ hour in an open dish.

It was not possible in this case to determine exactly the fixing power of the normal serum.   As a matter of fact in the experiments reported in this paper it is often necessary to examine the action of various pilocarpine solution 15 to 20 times on one and the same piece of gut and sometimes, before an exact standardisation of a solution is ready, the gut will fail to react.   This was the case in the experiment under discussion.   Notwithstanding that, the experiment is reported here because it could be clearly shown, that serum plus ether had a pilocarpine combining power 12, whereas a sample of serum plus ether, that had been kept in the ice-chest for one and one-half hours had a binding power 20.

Experiment VIII

October 8, 1920. This experiment was performed firstly to corroborate the findings of experiment VII and moreover we wanted to determine whether the binding of pilocarpine by rabbit serum, once being established could be subsequently loosened by the addition of ether.

a. Determination of the binding power of the normal serum used. 0.05 mgm. pilocarpine plus serum has a weaker action than 0.005 mgm. pilocarpine alone; 0.12 mgm. pilocarpine plus serum has a stronger action than 0.005 mgm. pilocarpine alone; 0.08 mgm. pilocarpine plus

serum has the same action as 0.005 mgm. pilocarpine alone.   Here then the·pilocarpine binding power was 16.

b. Determination of the pilocarpine binding power of serum plus ether.   0.004 mgm. pilocarpine plus serum plus ether has a weaker action than 0,003 mgm. pilocarpine alone; 0.00S mgm. pilocarpine plus serum plus ether has a stronger action than 0.003 mgm. pilocarpine alone.   Pilocarpine binding power of serum plus ether about 2.

c. Determination of binding power of serum plus ether after staying in the incubator at 37°C. for one and one-half hours.   0.02 mgm. pilocarpine plus serum plus ether (incubator) has a weaker action than 0.002 mgm. pilocarpine alone; 0.03 mgm. pilocarpine plus serum plus ether (incubator) has the same action as 0.002 mgm. pilocarpine alone; 0.04 mgm. pilocarpine plus serum plus ether (incubator) has a stronger action than 0.002 mgm. pilocarpine alone.   Pilocarpine binding power of serum plus ether (incubator) 15.

d. Determination of pilocarpine binding power of serum plus ether if first pilocarpine is added and only after three hours ether is added.   0.01 mgm. pilocarpine plus serum plus ether has a weaker action than 0.005 mgm. pilocarpine alone; 0.05 mgm. pilocarpine plus serum plus ether has a stronger action than 0.005 mgm. pilocarpine alone.   Pilocarpine binding power of the serum under these circumstances is more than 2, but certainly less than 10, which proves that the absorption of pilocarpine by rabbit serum can be loosened again by subsequent addition of ether.

The results then of this experiment are as follows:

a. Pilocarpine binding power of the normal rabbit serum used, 16.

b. Pilocarpine binding power serum plus ether, 2.

c. Pilocarpine binding power serum plus ether (after one and one-half hours at 37°C.), 15.

d. Pilocarpine binding power serum plus ether (addition of ether three hours after addition of pilocarpine), more than 2, less than 10.

In the experiments reported hitherto, we only wished to discover whether ether could decrease the pilocarpine binding power of serum at all, without considering the quantities of ether used. In most of the experiments we added such an amount that the serum contained about 0.3 per cent ether.   It was now necessary to make a quantitative study of the matter, and it was advisable to find out whether such quantities of ether as are present in the

blood during narcosis will influence the binding power of the serum.   With this point in view the next experiments were carried out.

Experiment IX

November 18, 1920. Determination of the pilocarpine binding power of the rabbit serum used.   0.12 mgm. pilocarpine plus serum has a weaker action, but nearly the same, as 0.03 mgm. pilocarpine alone; 0.3 mgm. pilocarpine plus serum has a stronger action than 0.03 mgm. pilocarpine alone.   Binding power of the normal serum 4.

Serum plus 2 per cent ether.   0.06 mgm. pilocarpine plus serum plus ether a little weaker action than 0.03 mgm. pilocarpine alone; 0.09 mgm. pilocarpine plus serum plus ether has a stronger action than 0.03 mgm. pilocarpine alone.   Binding power of serum plus 2 per cent ether about 2.

Experiment X

November 23, 1920. Pilocarpine binding power of normal serum used. 0.6 mgm. pilocarpine plus serum has a weaker action than 0.02 mgm. pilocarpine alone; 1 mgm. pilocarpine plus serum has a stronger action than 0.02 mgm. pilocarpine alone.   Binding power of the normal serum 30–50.

Serum plus 0.1 per cent ether.   0.08 mgm. pilocarpine plus serum plus 0.1 per cent ether has nearly the same action as 0.04 mgm. pilocarpine alone; 0.2 mgm. pilocarpine plus serum plus 0.1 per cent ether has a stronger action than 0.04 mgm. pilocarpine alone; 0.4 mgm. pilocarpine plus serum plus 0.1 per cent ether has much stronger action than 0.04 mgm. pilocarpine alone.   Pilocarpine binding power serum plus 0.1 per cent ether about 2.

Serum plus 1 per cent ether.   0.12 mgm. pilocarpine plus serum plus 1 per cent ether has a weaker action than 0.04 mgm. pilocarpine alone; 0.2 mgm. pilocarpine plus serum plus 1 per cent ether has a little weaker (nearly the same) action than 0.04 mgm. pilocarpine alone; 0.4 mgm. pilocarpine plus serum plus 1 per cent ether has stronger action than 0.04 mgm. pilocarpine alone.   Pilocarpine binding power of serum plus 1 per cent ether about 5.

Some phases of this experiment are reproduced in figure 2 a–d and figure 3 a–g.

In figure 2 a and 2 c 0.02 mgm. of pilocarpine is given to a

piece of isolated cat's intestine suspended in 75 cc. Tyrode solution.

In figure 2 b 0.6 mgm. pilocarpine plus normal serum (1 cc. of serum containing 1 mgm. of pilocarpine) is added.   The action is weaker than that of 0.02 mgm. pilocarpine alone.
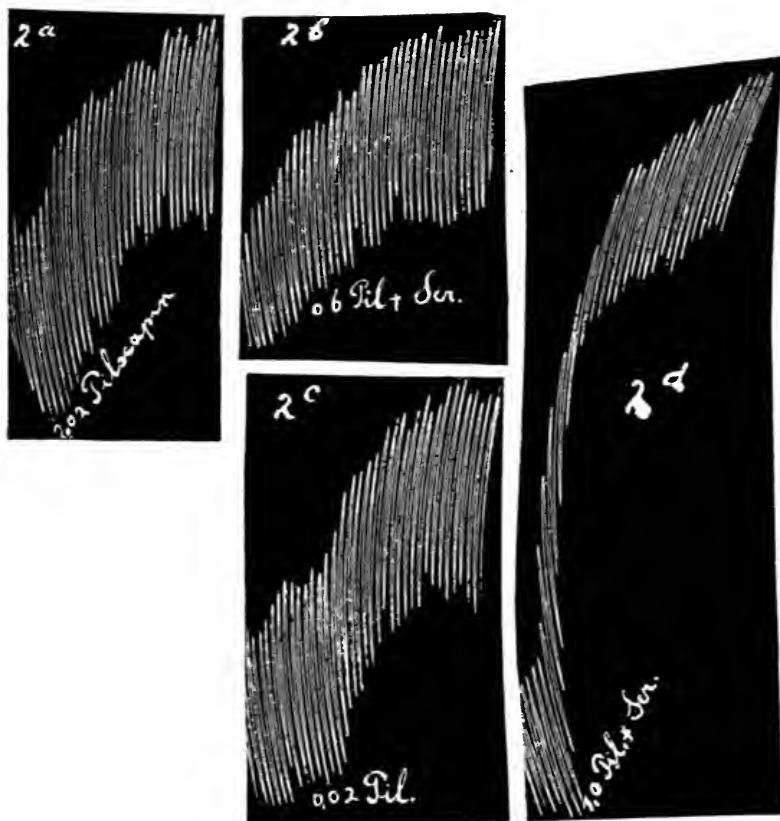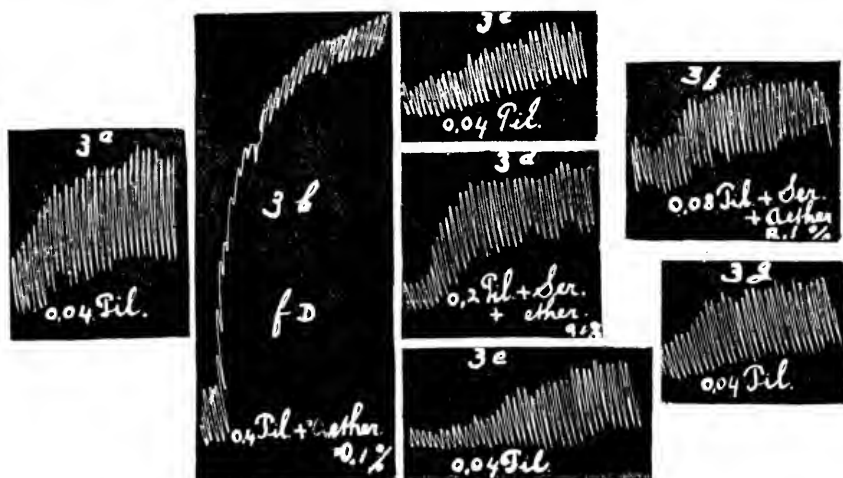

FIG. 2.   ISOLATED GUT SUSPENDED IN 75 C.C. TYRODE SOLUTION
a and c, action of 0.02 mgm, pilocarpine; b, 0.6 pilocarpine plus serum; d, 1 mgm. pilocarpine plus serum:

In figure 2 d 1, mgm. pilocarpine plus serum is given which produces a stronger action than 0.02 mgm. pilocarpine alone.

Figure 2 a–d then prove that the binding power of the normal rabbit serum used was more than 30 and less than 50.

In figure 3 a, 3 c, 3 e, and 3 g 0.08 mgm. of pilocarpine is added to the isolated gut.

In figure 3 b 0.4 mgm. pilocarpine plus serum plus 0.1 per cent ether is given. The contraction produced is stronger than that of 0.04 mgm. pilocarpine alone, showing that the binding power of serum plus 0.1 per cent ether is certainly less than 10.

In figure 3 d 0.2 mgm. pilocarpine plus serum plus 0.1 per cent ether is given. The action thereof is still slightly stronger than

FIG. 3. ISOLATED GUT SUSPENDED IN 75 CC. TYRODE SOLUTION.

a, c, e and g, action of 0.04 mgm. pilocarpine; b, action of 0.4 mgm. pilocarpine plus serum plus 0.1 per cent ether; d, action of 0.2 mgm. pilocarpine plus serum, plus 0.1 per cent ether.

f, action of 0.08 mgm. pilocarpine plus serum, plus 0.1 per cent ether.

that of 0.04 mgm. pilocarpine alone, so that the binding power must be less than 5.

In figure 3 f 0.08 mgm. pilocarpine plus serum plus 0.1 per cent ether produces a contraction almost the same as 0.04 mgm. before; the binding power of the serum plus 0.1 per cent ether can therefore be considered to be about 2.

Experiment XI ·

November 24, 1920. serum plus 0.5 per cent ether.

a. Determination of pilocarpine binding power of the rabbit serum used. 0.4 mgm. pilocarpine plus serum has weaker action than 0.04 mgm. pilocarpine alone; 0.08 mgm. pilocarpine plus serum has weaker (slightly) action than 0.04 mgm. pilocapine alone; 2 mgm. pilocarpine plus serum has much stronger action than 0.04 mgm. pilocarpine alone. Binding power of normal serum certainly not less than 20.

b. Serum plus 0.5 per cent ether. 0.04 mgm. pilocarpine plus serum plus 0.5 per cent ether has slightly weaker action than 0.02 mgm. pilocarpine alone; 0.08 mgm. pilocarpine plus serum plus 0.5 per cent ether has much stronger action than 0.02 mgm. pilocarpine alone; binding power serum plus 0.5 per cent ether about 2 (certainly much lower than 4).

Experiment XII

- *November 10, 1920.* Serum plus 0.2 per cent ether.

a. Binding power of normal serum used. 0.6 mgm. pilocarpine plus serum weaker action than 0.03 mgm. pilocarpine alone; 0.9 mgm. pilocarpine plus serum has nearly the same action as 0.03 mgm. pilocarpine alone. Binding power norma' rerum about 30.

b. Serum plus 0.2 per cent ether. 0.3 mgm. pilocarpine plus serum plus 0.2 per cent ether has a slightly stronger action than 0.3 mgm. pilocarpine alone; 0.4 mgm. pilocarpine plus serum plus 0.2 per cent ether has a distinctly stronger action than 0.03 mgm. pilocarpine alone. Binding power serum plus 0.2 per cent ether slightly below 10.

Quantitative determinations then of the influence of ether on the pilocarpine binding power show that

0.1 per cent ether could reduce the binding power from 30 (50) to 2 (X).

0.2 per cent ether could reduce the binding power from 30 to about 10 (XII).

0.3 per cent ether could reduce the binding power from 2 to 0 (V).

0.3 per cent ether could reduce the binding power from 16 to 2 (VIII).

0.5 per cent ether could reduce the binding power from 20 to 2 (XI).

1 per cent ether could reduce the binding power from 30 (50) to 5 (X).

2 per cent ether could reduce the binding power from 4 to ca. 2 (IX).

As it is known from former experiments (3), that during narcosis the blood of mammals contains from 0.1 to 0.17 per cent of ether it is obvious that the doses of ether, which were able in these experiments *in vitro* to reduce the pilocarpine binding power of rabbit serum, fall in the same range as the concentration of ether in the blood during narcosis.

After having studied the influence of ether in the way described it was desirable to study other narcotics; we chose chloroform, urethane and magnesium chloride.

Experiment XIII

November 15, 1920. Influence of chloroform (0.1 per cent).

a. Determination of the pilocarpine binding power of the rabbit serum used.   0.08 mgm. pilocarpine plus serum has a weaker action than 0.002 mgm. pilocarpine alone; 0.12 mgm. pilocarpine plus serum has a stronger action than 0.002 mgm. pilocarpine alone.   Binding power normal serum more than 40, less than 60.

b. Serum plus 0.1 per cent chloroform.   0.4 mgm. pilocarpine plus serum plus 0.1 per cent chloroform has a weaker action than 0.04 mgm. pilocarpine alone; 0.8 mgm. pilocarpine plus serum plus 0.1 per cent chloroform has nearly the same as 0.04 mgm. pilocarpine alone.   Binding power serum plus 0.1 per cent chloroform about 20.

Experiment XIV

November 16, 1920. Serum plus 0.1 per cent chloroform.

a. Determination binding power of serum used.   0.4 mgm. pilocarpine plus serum has a weaker action than 0.01 mgm. pilocarpine alone; 0.8 mgm. pilocarpine plus serum has a stronger action (nearly the same) as 0.01 mgm. pilocarpine alone.   Binding power normal serum 40–80.

b. Serum plus 0.1 per cent chloroform.   0.1 mgm. pilocarpine plus serum plus chloroform has a weaker action than 0.01 mgm. pilocarpine alone; 0.2 mgm. pilocarpine plus serum plus chloroform has a stronger action than 0.01 mgm. pilocarpine alone.   Binding power serum plus 0.1 per cent chloroform 10–20.

Experiment XV

November 24, 1920.

a. Binding power of serum used about 20 (same serum as used in experiment XI).

b. Serum plus 0.1 per cent chloroform. 0.12 mgm. pilocarpine plus serum plus 0.1 per cent chloroform has a weaker action than 0.04 mgm. pilocarpine alone; 0.2 mgm. pilocarpine plus serum plus 0.1 per cent chloroform has nearly the same action as 0.04 mgm. pilocarpine alone. Binding power serum plus 0.1 per cent chloroform about 5.

Experiment XVI

November 17, 1920. 0.4 per cent chloroform.

a. Binding power of normal serum used. 0.2 mgm. pilocarpine plus serum has weaker action than 0.02 mgm. pilocarpine alone, 0.4 mgm. pilocarpine plus serum has a slightly stronger action than 0.02 mgm. pilocarpine alone. Binding power normal serum more than 10 and lesss than 20.

b. Serum plus 0.4 per cent chloroform. 0.06 mgm. pilocarpine plus serum plus 0.4 per cent chloroform has a weaker action than 0.02 mgm. pilocarpine alone; 0.1 mgm. pilocarpine plus serum plus 0.4 per cent chloroform has a stronger action than 0.02 mgm. pilocarpine alone. Binding power serum plus 0.4 per cent chloroform certainly less than 5 and more than 3.

Experiment XVII

November 11, 1920. 1 per cent chloroform.

a. Binding power of normal serum used. 0.1 mgm. pilocarpine plus serum has nearly the same action as 0.003 mgm. pilocarpine alone; 0.12 mgm. pilocarpine plus serum has nearly the same action as 0.003 mgm. pilocarpine alone. Binding power normal serum about 33–40.

b. Serum plus 1 per cent chloroform. 0.02 mgm. pilocarpine plus serum plus 1 per cent chloroform has a slightly weaker action than 0.005 mgm. pilocarpine alone; 0.03 mgm. pilocarpine plus serum plus 1 per cent chloroform has nearly the same action as 0.005 mgm. pilocarpine alone; 0.04 mgm. pilocarpine plus serum plus 1 per cent chloroform has slightly stronger action than 0.005 mgm. pilocarpine alone. Binding power serum plus 1 per cent chloroform about 6 (certainly less than 8).

This experiment is reproduced in figure 4 a–d and figure 5 a–f.

In figure 4 a and 4 c 0.003 mgm. pilocarpine is given to a loop of intestine suspended in 75 cc. Tyrode solution. The action of this dose is distinctly stronger in figure 4 c than in figure 4 a illustrating the point described already in the first communica-
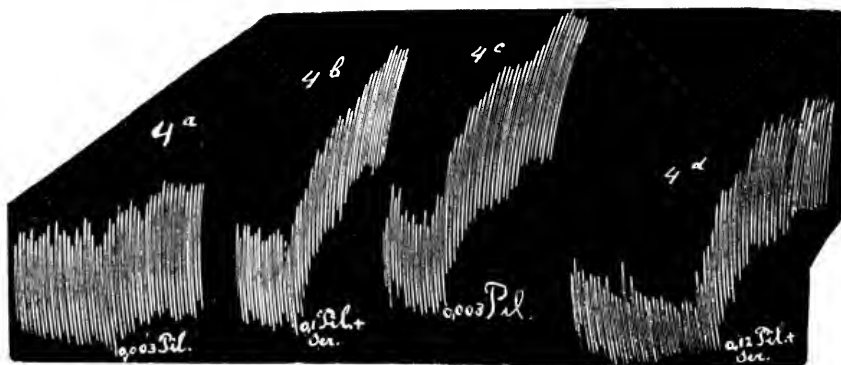
Fig. 4.   Isolated Cat's Intestine Suspended in 75 cc. Tyrode Solution

a and *c*, action of 0.003 mgm. pilocarpine (note difference in action in *a* and *c*, caused by the addition of serum in *b*); *b*, 0.1 mgm. pilocarpine plus serum; *d*, 0.12 mgm. pilocarpine plus serum.
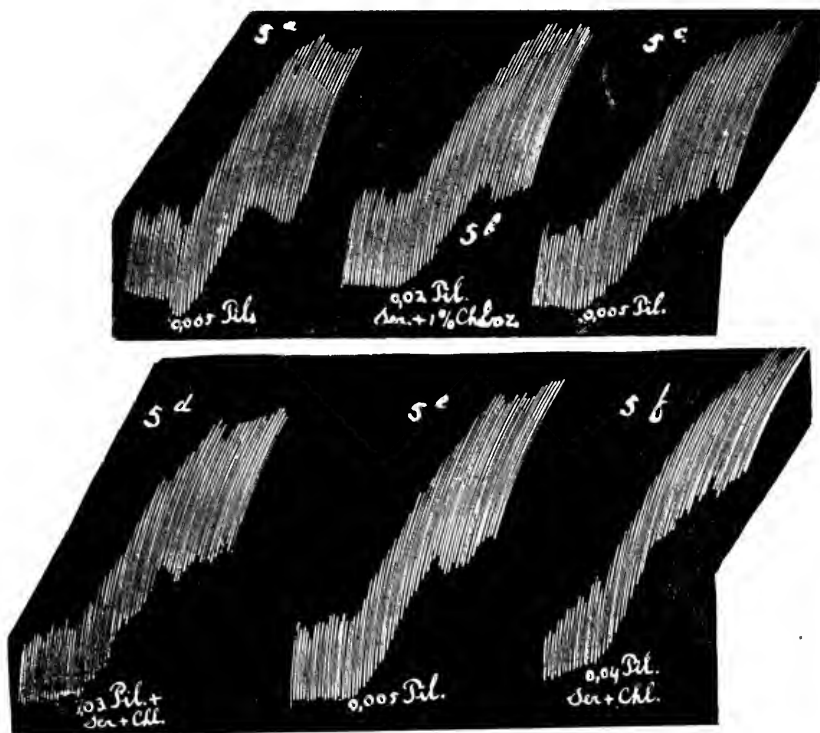

Fig. 5.   Isolated Cat's Intestine Suspended in 75 cc. Tyrode Solution.

a, *c* and *e*, action of 0.005 mgm. pilocarpine;   *b*, 0.02 mgm. pilocarpine plus serum plus chloroform 1 per cent;   *d*, 0.03 mgm. pilocarpine plus serum plus chloroform 1 per cent;   *f*, 0.04 mgm. pilocarpine plus serum plus chloroform 1 per cent.

284

tion, i.e., that very often after a dose of serum has been applied to the gut, the sensitiveness of the organ increases.

In figure 4 b 0.1 mgm. pilocarpine plus serum plus chloroform is given, in figure 4 d 0.12 mgm. pilocarpine plus serum plus chloroform. In both cases the contraction produced is nearly the same as in figure 4 a and 4 c.

In figure 5 a, 5 c and 5 e 0.005 mgm. pilocarpine is applied to the gut; the contractions produced thereby are almost exactly the same.

In figure 5 b 0.02 mgm. pilocarpine plus serum plus chloroform is given and in figure 5 d a slightly larger dose, viz., 0.03 mgm. pilocarpine. The difference in the action produced by these doses can hardly be seen, but 0.04 mgm. pilocarpine plus serum plus chloroform gives in figure 5 f a distinctly stronger contraction, showing that the binding power of the serum plus 1 per cent chloroform is certainly lower than 8.

Experiment XVIII

November 18, 1920.   1 per cent chloroform.

a. Pilocarpine binding power of the normal serum used 4 (same serum as used in experiment IX).

b. Serum plus 1 per cent chloroform.   0.06 mgm. pilocarpine plus serum plus 1 per cent chloroform has same action as 0.06 mgm. pilocarpine alone; 0.12 mgm. pilocarpine plus serum plus 1 per cent chloroform has a stronger action than 0.06 mgm. pilocarpine alone. Binding power serum plus 1 per cent chloroform 0.

Experiment XIX

November 16, 1920.   0.05 per cent chloroform.

a. Binding power of the normal serum used 40–80 (same serum as used in experiment XIV).

b. Serum plus 0.05 per cent chloroform.   0.2 mgm. pilocarpine plus serum plus 0.05 per cent chloroform has an action slightly stronger than 0.01 mgm. pilocarpine alone. Binding power serum plus 0.05 per cent chloroform about 20.

Experiments XIII to XIX then have shown, that chloroform can also inhibit the absorption of pilocarpine by rabbit serum,

though the doses required for this inhibition are larger, if the
narcotic action of both the drugs is taken into account, than
in the case of ether. Ether exerts a strong inhibition in con-
centrations which occur in the blood during narcosis, but in the
case of chloroform higher doses seem to be necessary. During a
chloroform narcosis of moderate depth the blood contains about
0.03 per cent chloroform (4); in very deep narcosis the percentage
may rise as high as 0.05 per cent. In the experiments described
above the values for the inhibition of pilocarpine at various
concentrations of chloroform are as follows:

0.05 per cent chloroform reduced the binding power from 40–80 to
20 (XIX).
0.1 per cent chloroform reduced the binding power from 40 to 20
(XIII).
0.1 per cent chloroform reduced the binding power from 40–80 to
10–20 (XIV).
0.1 per cent chloroform reduced the binding power from 20 to 5 (XV).
0.4 per cent chloroform reduced the binding power from 10–20 to
3–5 (XVI).
1 per cent chloroform reduced the binding power from 33–40 to
6–8 (XVII).
1 per cent chloroform reduced the binding power from 4 to 0 (XVIII).

This summary shows clearly, that even such low concentrations
of chloroform as appear in the blood during narcosis (0.05 per cent
are able to inhibit the pilocarpine absorption a little, but a strong
inhibition is only exerted by much larger concentration.

Experiment XX

*November 24, 1920. Influence of urethane 0.5 per cent on pilocarpine
binding power of rabbit serum.*

a. Binding power of normal serum used. 2.4 mgm. pilocarpine plus
serum has a weaker action (nearly the same) than 0.06 mgm. pilocarpine
alone. Binding power normal serum about 40.

b. Serum plus urethane 0.5 per cent. 0.8 mgm. pilocarpine plus serum
plus 0.5 per cent urethane has weaker action (nearly the same) than
0.02 mgm. pilocarpine alone. Binding power 0.5 per cent urethane
about 40.

Urethane then in a concentration much larger than can be expected in the blood of an animal during urethane narcosis has no influence at all on the binding power of rabbit serum. In the next experiment urethane exerted a slight inhibition.

Experiment XXI

Serum plus 0.5 per cent urethane.
Binding power of normal serum 40–60.
Binding power of normal serum plus 0.5 per cent urethane 15–30.

These experiments were not continued since it soon appeared that smaller concentrations of urethane have no effect at all on the binding power of the serum, whereas larger doses will *per se* reduce the contractions of the isolated gut, so that experiments with these concentrations are impossible.

The same can be said of our experiments with magnesium sulphate.

Experiment XXII

Influence of 0.4 per cent magnesium sulphate on pilocarpine binding power of rabbit serum.
Binding power of normal serum used 20–40.
Binding power of normal serum plus 0.4 per cent magnesium sulphate 20–40.

This concentration of magnesium sulphate, then, had no influence on the pilocarpine absorption, but the experiment is not entirely conclusive as the dose of magnesium sulphate used might have slightly inhibited the contractions of the gut. For the reason stated, the experiments with urethane and magnesium sulphate were discontinued, and we are only in a position to state, that small doses such as occur in the blood of an animal during narcosis have no influence. The effect of larger concentrations could not be studied.

As it had been shown in the 2nd communication, that the binding of atropine by rabbit serum can be loosened by the addition of small quantities of peptone, we were interested to know whether peptone would be able also to exert an influence on the pilocarpine absorption.

Experiment XIII

Influence of peptone (Witte) on pilocarpine binding power of rabbit serum.

a. Binding power of the normal serum used. 0.004 mgm. pilocarpine plus serum has a weaker action than 0.002 mgm. pilocarpine alone; 0.006 mgm. pilocarpine plus serum has a stronger action than 0.002 mgm. pilocarpine alone. Binding power serum 2–3.

b. Serum plus 0.5 per cent peptone (Witte). 0.003 mgm. pilocarpine plus serum plus 0.5 per cent peptone has same action as 0.003 mgm. pilocarpine alone; 0.004 mgm. pilocarpine plus serum plus 0.5 per cent peptone has a stronger action than 0.003 mgm. pilocarpine alone. Binding power serum plus 0.5 per cent peptonenil.

c. Serum plus 0.1 per cent peptone. 0.004 mgm. pilocarpine plus serum plus 0.1 per cent peptone has same action as 0.004 mgm. pilocarpine alone; 0.005 mgm. pilocarpine plus serum plus 0.1 per cent peptone has a stronger action than 0.004 mgm. pilocarpine alone. Binding power serum plus 0.1 per cent peptonenil.

Experiment XXIV

In this experiment the serum used had only a very slight binding power for pilocarpine, addition of peptone to make a concentration of 0.5 per cent and of 0.1 per cent did not have much influence. Therefore we tried to investigate the matter in further experiments. We had however to give up these attempts, as the results obtained in the different experiments varied widely. The reason is to be found in the fact that, as had been pointed out by one of us before (1), peptone can *per se*, according to the mode of procedure followed in the experiments, either inhibit or augment the action of pilocarpine.

We thought it worth while however to investigate some other colloids. We tried starch and lecithin, and both of them were entirely negative in this respect as will be seen in the following protocols.

Experiment XXV

October 6, 1920. Serum plus starch.

a. Binding power of normal serum. 0.4 mgm. pilocarpine plus serum has a weaker action than 0.02 mgm. pilocarpine alone; 0.8 mgm. pilocarpine plus serum has a stronger action than 0.02 mgm. pilocarpine alone. Binding power serum about 20.

b. Serum plus starch 0.5 per cent 0.2 mgm. pilocarpine plus serum plus starch has a weaker action than 0.02 mgm. pilocarpine alone. Binding power serum plus starch 0.5 per cent about 20.

c. Serum plus 0.1 per cent starch. 1 mgm. pilocarpine plus serum plus 0.1 per cent starch has much weaker action than 0.1 mgm. pilocarpine alone. Binding power certainly much higher than 10.

Experiment XXVI

October 7, 1920. Serum plus 2 per cent lecithin.

a. Binding power of normal serum used. 0.04 mgm. pilocarpine plus serum has weaker action than 0.005 mgm. pilocarpine alone; 0.05 mgm. pilocarpine plus serum has same action as 0.005 mgm. pilocarpine alone; 0.08 mgm. pilocarpine plus serum has a stronger action than 0.005 mgm. pilocarpine alone. Binding power serum 10.

b. Serum plus 1 per cent lecithin. 0.03 mgm. pilocarpine plus serum plus 1 per cent lecithin has a slightly weaker than 0.003 mgm. pilocarpine alone. Binding power serum plus 1 per cent lecithin about 10.

CONCLUSION

The research reported here was started with the purpose of finding an explanation for the differences in the binding power of sera of various animals. It may be stated at once, that we have not succeeded in clearing up the problem entirely. Even if every influence that can be controlled is kept constant, very large differences between different animals are found. One source of error however revealed itself distinctly during our work, i.e., the influence of narcosis on the pilocarpine fixing power of the serum. We found that the absorption of pilocarpine by rabbit serum can be inhibited by chloroform and still more strongly by ether in concentrations which occur in the blood during narcosis. Peptone has a similar effect, but urethanel, magnesium sulphate, starch and lecithin have no influence in this respect.

The inhibiting action of peptone on the absorption of pilocarpine is not an isolated phenomenon. One of us showed in collaboration with Zeydner, that peptone also inhibits the absorption of atropine by rabbit serum (2). Inhibition of absorption processes by various colloids and other substances has been

studied also by Gengou (5) who found among several other facts, that the absorption of dyes by animal charcoal can be inhibited by citrate solutions. Finally it is known, as already mentioned above, that the formation of anaphylatoxin in vitro can also be inhibited by the addition of ether and other narcotics.

We are of the opinion, that the importance of the fact that ether can inhibit pilocarpine absorption is not limited to the study of differences in binding power of several sera, but that it is more far-reaching.

In a former publication we pointed out, that the formation of anaphylatoxin in vitro by addition of agar-agar, gelatine and so forth, to guinea-pig serum may depend on changes in the relation between the various colloids in the serum, through which change the action of poisons already present in the serum may be intensified. The experiments reported in the present paper suggest another possibility. If an innocuous serum (rabbit serum plus pilocarpine or rabbit serum plus atropine) can become highly poisonous by the mere addition of peptone (which loosens the binding of pilocarpine and atropine to serum substances), it is conceivable that similar phenomena occur when innocuous guinea-pig serum becomes poisonous by the addition of agar-agar, or gelatine or inulin. How far one or both of the hypotheses offered here for the formation of anaphylatoxin in vitro is supported by experimental evidence will be studied in this institute in the near future.

Influence of colloids on the action of drugs—as shown to occur in vitro—will very likely play a part also in vivo.

It is beyond doubt that under physiological conditions various poisons occur in animal blood. It is, in our opinion, highly probable that the action of these poisons will be influenced by the various colloidal substances present in the serum; this influence may be inhibitory or augmentor. If by the mere addition of a small quantity of ether to a mixture of serum plus pilocarpine the action of this mixture on the isolated intestine can be increased many times (by the inhibiting action of the ether on the pilocarpine absorption) is it not probable then, that similar processes may occur in the animal body during narcosis?

Recently Dale (6) published interesting researches on the toxicity of histamine for the cat. He found that histamine is about 10 times more poisonous for the narcotized cat than for the normal animal. We think it quite possible that this phenomenon can be explained by the assumption that the ether has in Dale's experiments an action similar to that exerted in our pilocarpine experiments, i. e., the ether may increase the toxicity of the histamine simply by changing relations between the various colloids in animal blood. Preliminary experiments that were made to test this hypothesis seemed to give an indication that our assumption may be right. The results of this work will be published in a later publication.

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THE SENSITIVENESS TO POISONS IN AVITAMINOUS ANIMALS

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Since Eykman found in 1893 that fowls fed on a diet of polished rice only would after some weeks show signs of "polyneuritis gallinarum" and that this disease could be cured by feeding the animals with the bran of the rice, a large amount of work in deficiency diseases has been performed. As a result of this work, which during the last years was taken up with great zeal by American investigators, our knowledge of the factors determining the outbreak of deficiency diseases and our knowledge of the several accessory foodstuffs, fat soluble A, water soluble B and so forth has been very much increased. On a certain point however—and a very important point too—we are still groping in the dark. We know that by giving to animals a diet lacking in certain substances, these animals will show after a certain time definite symptoms of illness, but the question as to what is finally the cause of the illness in these animals is not yet solved. There is a theory that withdrawal of certain foodstuffs from the diet merely increases the sensitiveness of the animal for bacterial infection but this theory, if being supported at all by experimental data, can certainly not give an adequate explanation for all cases of deficiency diseases. There are theories that ascribe the symptoms of deficiency diseases to the formation of a "poison" in the body of the avitaminous animals, but of the nature of this hypothetical poison nothing is known.

McCarrison (1) in a very extensive study on deficiency diseases and accessory foodstuffs supports the view that the lack of vitamines induces above all a defect in "matière nucleaire" in

the body, which defect the body tries to meet by reducing the amount of that material in several organs.

Recently Uhlmann (2) found that a vitamine preparation "orypan" (and also other vitamines) contain substances which have an action on smooth muscles very closely resembling that of pilocarpine and previous to this work it had already been shown by Bickel (3) that extracts of spinaches exert a pilocarpine-like action. On the bases of these findings it would be possible to ascribe many of the deficiency symptoms to a lack of "pilo-carpine-like" substances which stimulate smooth musculature. Such a view would be in accordance with the opinion expressed by Hopkins, Abderhalden, Bang and others, who also seem to incline to consider the vitamines as being stimulants for cell activity.

One of us (V.) had in collaboration with Bögel (4) and inde-pendently of Uhlmann's work studied the influence of extracts from foodstuffs containing either fat soluble A or water soluble B, on isolated muscle and isolated gut, on the isolated rabbit's ear and on the Laewen Trendelenburg preparation of the frog, on blood pressure in dogs and on the sugar excretion in pancreas diabetic dogs. They could in certain instances find a slight action of their extracts, but they did not feel justified to ascribe this pharmacological action to the vitamin fraction of their extracts.

As however Verzár and Bögel were through external circum-stances during the performance of the experiments related above, not in a position to carry out metabolism experiments they were unable to verify the "vitamine" action of their extracts and moreover the extracts used by them were derived from another source than those of Uhlmann. The present writers are of the opinion that Uhlmann's results are not disproved by the experi-ments of Verzár and Bögel.

Although, as stated above, a theory ascribing the curative effect of vitamines in deficiency diseases could to a certain degree be supported by the results of some experiments, we are of the opinion that this question cannot at all be considered as being settled. Since work that had been done by one of us (S. v. L.) seemed to open the possibility of coming a little nearer to the

solution of the question under discussion we felt justified in undertaking a series of experiments which will be reported here.

The starting point of our work was determined by the following considerations.

Among the symptoms exhibited by animals suffering from deficiency diseases, disturbances in the action and in the innervation of striped and smoothe muscles play a large part.   It is known that these disturbances in the function of muscles can be to some extent explained by the fact that signs of extensive degeneration in nerves and muscles are to be found.   The degeneration in the spinal nerves in cases of avitaminose has long been known.   That also in these cases degenerative signs can be demonstrated to a large extent in the neuromuscular system of the gut and the other visceral organs was recently emphasized by McCarrison (1).

These organic lesions in nerves, muscles and secretory organs can however never explain the entire complex of symptoms in avitaminoses, since it is possible to cure an animal suffering from polyneuritis in an extraordinary short time.   Hence it must be assumed that the symptoms of deficiency diseases are at least partly of functional nature, i.e., the organs of the animal do not react to the stimuli being present in the body at that time, but these organs can be brought into action quasi at once by the injection of the adequate vitamine.

If now it is asked why those muscles (and in the present paper we will only consider the smooth muscles), did not react properly before the vitamine was injected it seems to us that from a theoretical standpoint three solutions of the question might be deemed possible.

a. The smooth muscles of the organs do not react because substances which under physiological conditions are the normal stimulants for these organs are during the deficiency diseases not present in adequate amounts.

b. The smooth musculature does not react because its sensitiveness to stimulating substances, present in normal amount, has been lowered.

c. The sensitiveness of smooth musculature is normal, also the

amount of stimulating principles is normal, but there is a lack of (colloidal) substances in the body of the animal which under normal conditions tend to facilitate the action of drugs on smooth musculature.   That colloidal substances really occur under normal conditions in the animal body had been shown by one of the present writers in corroboration with his co-workers (5, 6, 7) and we were especially interested to find out whether some . symptoms of deficiency diseases would be caused by a lack of these substances.   Although this last mentioned assumption could be proved not to be true, we deemed it advisable to publish our results as it appears to us that the question of the ultimate cause of the symptoms in avitaminosis cannot be solved, and the importance which Uhlmann's researches may have in this matter cannot be rightly valued before it is known which of the three possibilities mentioned above is the right one.

The immediate aim of our experiments was to make out whether in deficiency diseases a diminished or anyhow a changed sensitiveness of the smooth musculature to drugs could be demonstrated. If a change in sensitiveness had been found it would of course have been necessary to differentiate between the sub b and c, mentioned possibilities.

We performed our experiments on fowls and cats.   Deficiency diseases were induced in the fowls by feeding them with polished rice for some weeks whereas the cats were fed exclusively on meat which after having been made alkaline had been heated in the autoclave for three hours at 120°.   This last method which has been described by Voegtlin and Lake (8) gave us good results.

We are of course aware of the fact that the food we gave our animals in these experiments was not only lacking in one certain vitamine but was deficient in many respects but on account of what is known in the literature it could be assumed that the symptoms which our animals showed would be mainly dependent on a lack of water soluble B.   This question however was immaterial to us since we first had to investigate whether there could be demonstrated any differences at all in the reaction of normal and avitaminous animals.   Only if this had been proved to be the case it would have been necessary to differentiate between the effect of the omission of different vitamines.

The avitaminous animals were only experimented upon when they showed very markedly the characteristic symptoms of polyneuritis; as a matter of fact some of the animals were nearly moribund during the experiments so that it was hardly possible to narcotize them.   In most cases we used very light ether narcosis and some times urethane narcosis.   A cannula was tied in the carotids for measurement of the blood pressure (with Hg manometer) and another cannula in the vena femoralis in cats and in one of the veins of the wing in fowls to make the injections with the various drugs.   The vagi were prepared free near the larynx, tied and cut.   We then determined the minimum dose of adrenaline, choline and histamine which by intravenous injection just gave a distinct but slight action on the blood pressure; in many cases also the influence of larger doses was tried.   All drugs were given in such a dilution that 0.5 to 1 cc. had to be injected. The injection always lasted exactly twenty seconds.   After the sensitiveness for the drugs mentioned had been tested we determined the minimal strength of electric current which on stimulation of the vagus nerve gave a distinct fall of blood pressure, and after that we tried to find the minimal amount of atropine which could inhibit this vagus action.   When the blood pressure experiment was finished, the animal was killed and the gut removed.   Isolated strips of gut were then suspended in Tyrode solution and the sensitiveness to pilocarpine, histamine, choline and atropine was tested.   Sometimes we also used strips of the esophagus in these experiments.

As the sensitiveness of normal fowls to drugs was not known to us, we had to make preliminary determinations on them.   The results of these experiments are included in the present paper. It was hardly necessary to make control experiments on cats since the normal reactions to the drugs to be tested were known to us.

EXPERIMENTS ON FOWLS

Normal fowls.   The reaction of 5 normal fowls was studied. The exact data obtained in these experiments will be given in the tables below.   The blood pressure at the beginning of these experi-

ments varied from 110 to 152 mm. Hg. Very small doses of adrenaline viz., 0.0005 mgm. adrenaline (Parke, Davis and Company) dissolved in 1 cc. of NaCl solution and injected at a constant rate in twenty seconds gave generally a drop in blood pressure; slightly larger doses gave a rise, preceded or followed by a drop, whereas doses of 0.001 to 0.005 mgm. generally gave a rise in blood pressure. The sensitiveness of the various animals to adrenaline differed widely. Fowl II reacted to 0.01 mgm. of adrenaline with a drop of 12 mm. Hg followed by a rise of 16 mm. Hg whereas fowl I gave a drop in blood pressure of 24 mm. Hg after injection of 0.0005 mgm. adrenaline. It was often difficult to determine exactly the minimum active dose of adrenaline, so the figures given in table 1 are approximate ones and indicate the doses after which a *distinct* rise of blood pressure following the adrenaline injection was seen independently of whether this rise was preceded or followed by a fall in blood pressure. It was thought at first that it would be of advantage to differentiate exactly between doses that would give a fall and doses that would give a rise, as we deemed it possible that avitaminous animals would behave differently in this respect. This however proved not to be the case. Also in the fowls suffering from deficient diet we noted sometimes a rise and sometimes a drop of blood pressure after small doses of adrenaline. As moreover even in the same animal the reaction to adrenaline was not a constant one, since the same dose of adrenaline, which gave a rise of blood pressure before might cause a drop of pressure half an hour later, we left these differences out of consideration and give in table 1 those doses which caused a distinct rise in blood pressure.

Choline. A study of the action of choline on the blood pressure seemed very promising to us as choline belongs to the normal constituents of the blood. The result however was disappointing. The doses that may give a distinct drop in blood pressure vary widely in different animals. We often found a reaction after small doses, but fowl VI for instance failed to react on 2 mgm. of choline. So we gave choline injections only in a few cases. As far as we could make out there are no differences in this respect

between the behaviour of normal fowls and of animals suffering from avitaminosis.

Histamine. In the beginning of our experiments we used a sample of ergamine obtained by the kindness of Dr. Dale. Later we used the same preparation obtained from Burroughs and Welcome. These ergamine experiments gave very clear results. 0.01 mgm. of ergamine always gives a distinct drop in blood pressure (15 to 20 mm. Hg) sometimes smaller doses were also active. In table 3 we give only one instance of histamine action in normal fowls. In another series of experiments however on normal fowls in this laboratory it was found that 0.01 mgm. of ergamine is always active.

Action of atropine on effect of vagus stimulation

It is of course not possible to determine in every single experiment the exact minimal dose of atropine which will inhibit the effect of vagus stimulation. One has to try a very small dosis first and if this one is not sufficient a higher dose is given, till a dose is found which inhibits the vagus effect. But with this mode of procedure it is not known whether in determining the effective dosis all the single doses have to be added or not. Presumably part of the atropine injected with the first doses will be destroyed or will be bound somewhere in the body at the moment the last dosis is given. On the other hand it is known that the action of atropine, once being established lasts for a considerable time so that certainly part of the atropine of the first injections will add its effect to that of the later injections. We considered as an active dose of atropine the sum of the small doses given till the vagus stimulation had no influence on the blood pressure. In this way we found as effective doses of atropine 0.01 to 0.02 mgm.

Action on isolated gut

It was known from former experiments that the sensitiveness of pieces of catgut suspended in Tyrode solutions to pilocarpine, histamine and so forth, varies greatly in different pieces even if they belong to the same animal. Moreover the sensitiveness of

a certain loop is by no means constant during the course of an experiment; usually there is a tendency to an increased sensitivity as the experiment proceeds. Hence it was not advisable to try to find exact minimum active doses for the drugs under investigation. We knew from a great many experiments on isolated catgut in Tyrode solution that this organ will, as a rule, react to 0.005 to 0.01 mgm. of pilocarpine hydrochloride added to 75 cc. of Tyrode solution. The effect of a dose of pilocarpine, which gives a marked contraction of the gut can be influenced antagonistically by 0.0001 to 0.001 mgm. of atropine (7). As it appeared after the first experiments with the isolated gut of fowls that also in the organs of this animal great differences in susceptibility are to be found, we contented ourselves with the investigation whether the sensitiveness of the gut of the fowl would be about the same as that of the cat. This indeed proved to be the case as in several experiments 0.005 to 0.01 mgm. of pilocarpine was active (in one case also 0.001 mgm.) whereas this action could be influenced antagonistically by doses of atropine corresponding to the above-mentioned active doses for the cat.

Histamine was active on the gut of the fowl in doses of 0.01 to 0.05 mgm. ergamine added to 75 cc. Tyrode solution. These doses fall into the same range as those found in the cat.

Choline was active on the gut of the fowl in doses of 0.05 to 1 mgm. added to 75 cc. Tyrode solution. It was not attempted to determine the exact minimal active doses.

On the *isolated esophagus* of the fowl only a few experiments were performed. In one experiment it reacted to 0.1 mgm. of pilocarpine added to 75 cc. Tyrode solution with a fairly strong contraction whereas the contraction was inhibited by 0.01 mgm. of atropine (smaller doses would presumably have been active also but they were not tried).

Experiments on fowls suffering from avitaminosis

Investigations were made on 9 fowls. They were not experimented upon until the symptoms of polyneuritis were fully developed. Some animals were so ill at the beginning of the experiment that it was hardly possible to narcotize them. For

this reason it was not possible to make all the necessary determinations on every animal because sometimes they died during the experiment.

Blood pressure. The blood pressure at the beginning of the experiment was as a rule lower in the animals suffering from avitaminosis than in the normal ones. In 3 cases very low pressures were found (50 mm. of Hg at the beginning and down to 10 to 20 mm. at the end of the experiment) but in other cases we found 140 to 145 mm. Hg.

Fig. 1. Influence of Adrenaline (Parke, Davis and Company) on Blood Pressure of a Fowl Suffering from Avitaminosis

Blood pressure 90 mm. of Hg. 0.0005 mgm. of adrenaline dissolved in 1 cc. of NaCl solution and injected in twenty seconds with constant rate intravenously gives very slight reaction. 0.001 mgm. causes slight rise in blood pressure. 0.005 mgm. gives moderate rise in blood pressure.

The blood pressure could be experimentally raised by stimulation of a sensible nerve (ischiadus f. i.). Stimulation of the vagus with currents of the same strength as in the normal gave a definite fall in pressure.

Action of adrenaline. Exact information on the adrenaline action could not in all cases be obtained, as in those cases where

the blood pressure was very low, there was a continuous tendency to clotting. One animal (fowl VIII) seemed to be very resistant to adrenaline. The blood pressure however was low at the beginning of the experiment (50) and went down to 20 mm. Hg during the first ten minutes, in fact this animal was moribund so that, in view of the results obtained in other animals, we incline to disregard this experiment. In 5 cases where exact determination could be made the active dosis varied from 0.0003 mgm. to 0.001 mgm. of adrenaline dissolved in 1 cc. and injected into the vena femoralis at a constant rate, the entire injection taking exactly twenty seconds. Figure 1 gives an instance of the action of 0.0005 mgm. of 0.001 mgm. and of 0.005 mgm. of adrenaline in the blood pressure of fowl V suffering severely from polyneuritis at the time of the experiment.

Choline was active in one case in doses of 0.2 to 2 mgm., but as stated above we do not lay much stress on the cholin experiments.

Histamine. Ergamine was active in all the cases (6) where it was tried in doses of 0.01 to 0.05 mgm. In one case a drop in blood pressure was obtained with 0.002 mgm.

Atropine on effect of vagus stimulation. The atropine dosis necessary to inhibit the effect of vagus stimulation was determined in 3 cases. The doses were 0.005 mgm., 0.01 mgm. and 0.1 mgm. It is not certain whether in the last mentioned case a smaller dosis would not also have been sufficient.

Action on isolated gut

The action of pilocarpine on the isolated gut was tested in 8 cases. The active doses varied from 0.001 to 0.01 mgm. pilocarpine hydrochloride added to 75 cc. Tyrode solution.

Atropine. The inhibitory effect of atropine on pilocarpine contraction was studied in 8 cases; the active doses varied from 0.0001 to 0.001 mgm. of atropine sulfate added to 75 cc. of Tyrode solution.

In one case (fowl VI) 0.00005 mgm. was also active.

Figure 2 a gives an instance of the action of 0.005 mgm. of pilocarpine on the gut of a normal fowl suspended in 75 cc. Tyrode solution; the action is inhibited by 0.0001 mgm. of atropine sulfate.

Figure 2 b gives the action of 0.01 mgm. of pilocarpine in the gut of a fowl (V) suffering severely from polyneuritis. The pilocarpine action is inhibited by 0.0001 mgm. of atropine.

Histamine. Ergamine was tried in 3 cases; it was active in doses of 0.02 to 0.05 mgm. In one case (IX) only 0.1 mgm. of

FIG. 2A. INFLUENCE OF PILOCARPINE HYDROCHLORIDE AND ATROPINE SULFATE ON ISOLATED GUT OF NORMAL FOWL

0.005 mgm. of pilocarpine added to 75 cc. Tyrode solution in which the gut is suspended gives contractions which are inhibited by 0.0001 mgm. of atropine.

FIG. 2B. INFLUENCE OF PILOCARPINE AND ATROPINE ON ISOLATED GUT OF FOWL SUFFERING FROM EXPERIMENTAL POLYNEURITIS

ergamine was tried. It was very active. Smaller doses were not tested in this experiment.

Figure 3 a gives an instance of the action of 0.05 mgm. of ergamine added to the 75 cc. of Tyrode solution in which the isolated piece of gut of a normal fowl was suspended.

Figure 3 b gives the action of the same dosis of ergamine on a piece of gut of a fowl suffering from polyneuritis. This action can easily be inhibited by 0.01 mgm. of atropine. Very likely smaller doses would have been sufficient in this case.

Choline. Choline was given to three pieces of gut; 1 and 2 mgm. were active.
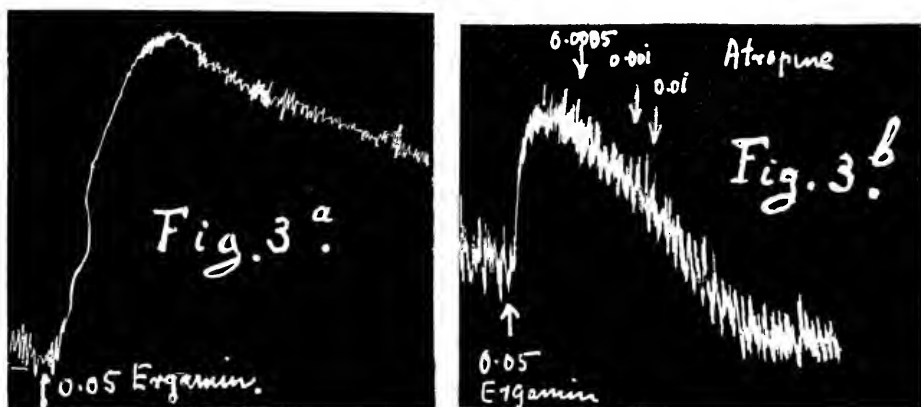
FIG. 3A. ACTION OF 0.05 MGM. OF ERGAMINE (BURROUGHS, WELCOME AND COM-
PANY) ON ISOLATED GUT OF NORMAL FOWL
FIG. 3B. ACTION OF 0.005 MGM. OF ERGAMINE ON ISOLATED GUT OF FOWL SUF-
FERING FROM EXPERIMENTAL POLYNEURITIS
Action of ergamine inhibited by atropine

Action on the esophagus

Pilocarpine in doses of 0.1 to 0.15 mgm. gave contraction of a isolated piece of esophagus in all the 4 cases where it was tried. 0.001 to 0.01 mgm. of atropine inhibited this pilocarpine contraction.

Figure 4 gives the action of 0.1 mgm. of pilocarpine on an isolated piece of the esophagus of a fowl suffering from poly-neuritis; this pilocarpine action is partly inhibited by the addition of 0.001 mgm. of atropine. The complete data of the experiments related here are given below in tables 1 to 11.

From these tables and from the description given above it will be clear that the result of our investigation is an entirely negative one. As stated above, the sensitiveness of different individuals and even of different pieces of isolated organs of the

same individual—to the drug used varies greatly. Hence it is very difficult to get very exact values for the minimum active doses, and we agree that a slight difference that might exist between the normal fowls and those suffering from polyneuritis might have escaped our attention. We are of the opinion however that if the very severe symptoms that all our polyneuritis cases showed had been caused by a lack of susceptibility to stimulating chemical agents, very great differences in sensitiveness were to have been expected. And great differences were certainly absent in our cases.
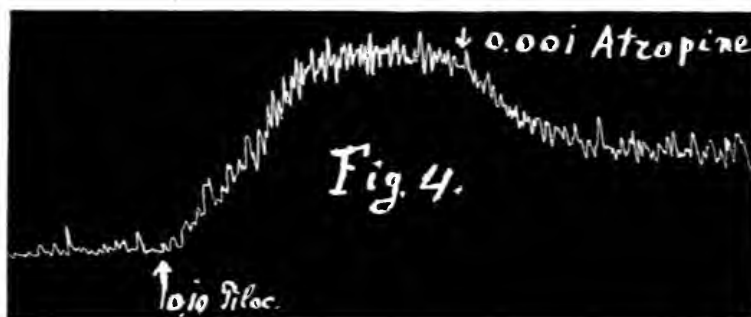
FIG. 4. ACTION OF 0.1 MGM. OF PILOCARPINE ON ISOLATED ESOPHAGUS OF FOWL SUFFERING FROM EXPERIMENTAL POLYNEURITIS

Pilocarpine action partly inhibited by 0.001 mgm. atropine

TABLE 1

Blood pressure at the beginning of the experiment

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	135	152	145	128	110	—	—	—	—
Polyneuritis cases..	145	140	124	54	90	—	140	50(20)	145

TABLE 2

Minimum active dose of adrenalin, in milligram of adrenaline (Parke, Davis and Company). Dissolved in 1 cc., injected in vena femoralis in 20"

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	0.0005	0.005	0.001	0.005	0.0005	—	—	—	—
Polyneuritis cases..	0.001	0.0003	0.001	—	0.0003	—	0.003	0.005*	0.0005

* Very slight reaction. The animal did not react to further doses of adrenaline and died soon afterwards.

TABLE 3

Doses of ergamine in milligram (Burroughs, Welcome and Company) which give definite fall in blood pressure

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	—	—	—	0.01*	—	—	—	—	—
Polyneuritis cases..	—	—	—	0.05	—	—	0.01	—	0.002
									0.02

* In 3 other cases not belonging to this series the same dose of ergamine was active.

TABLE 4

Effective dose of choline on blood pressure in milligrams.

Normal fowls... 2.0
Polyneuritis fowls... 0.2 to 2.0

TABLE 5

Inhibition of effect of vagus stimulation—minimal effective atropine doses in milligram

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	—	0.01	0.01	0.01	0.02	—	—	—	—
Polyneuritis cases..	—	0.01	0.1	—	—	—	—	—	0.005

TABLE 6

Minimal effective dose of pilocarpine in milligram on isolated gut

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	—	—	0.005	0.01	0.001	—	—	—	—
Polyneuritis cases..	0.02	0.001	0.05	—	0.001	0.005	0.01	0.01	0.01

TABLE 7

Minimal dose of atropine in milligram which inhibits pilocarpine action

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls......	—	—	0.0001*	—	—	—	—	—	—
Polyneuritis cases.	0.001	0.001	0.001	—	0.0001	0.00005	0.0001	0.0001	0.001

* In a large series of normal cases on cat min. effective dose 0.0001 to 0.005 mgm. atropine.

TABLE 8

Effective dose of ergamine in milligrams on isolated gut

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	—	—	—	0.05	—	—	—	—	—
Polyneuritis cases..	—	—	—	0.02	—	—	0.05	—	0.1

TABLE 9

Effective dose of choline in milligrams on isolated gut

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	0.05	1.0	—	—	—	—	—	—	—
Polyneuritis cases..	—	—	.	1.0	—	2.0	—	—	2.0

TABLE 10

Effective dose of pilocarpine in milligram on isolated esophagus

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	—	—	0.1	—	—	—	—	—	—
Polyneuritis cases..	—	0.1	0.15	—	0.1	—	—	0.15	—

TABLE 11

Dose of atropine in milligram which inhibits pilocarpine action on isolated piece of esophagus

	I	II	III	IV	V	VI	VII	VIII	IX
Normal fowls.......	—	—	0.01	—	—	—	—	—	—
Polyneuritis cases..	—	0.01	0.005	—	0.005	—	—	0.001	—

Experiments on cats

The experiments on cats were performed in the same way as those on fowls. The animals were only fed on meat heated for three hours in the autoclave at 120°C. (Voegtlin and Lake (8)). Marked symptoms of polyneuritis were present fifty to sixty days after the beginning of this diet. The cats were not used for the experiment until they were very ill; two animals died on the operating table so that only the sensitiveness of the isolated organs could be studied. With two cats we were able to make

a complete investigation of the susceptibility to drugs. As it was noted that in these 4 animals there was (as with the fowls), no change in sensitiveness to drugs demonstrable (with one exception to be mentioned below) we abandoned further experiments.

. A short description of the investigation on cats will be given here.

Blood pressure. The blood pressure at the beginning of the experiment was low in both cases (very light ether narcosis), 66 and 70 mm. of Hg.
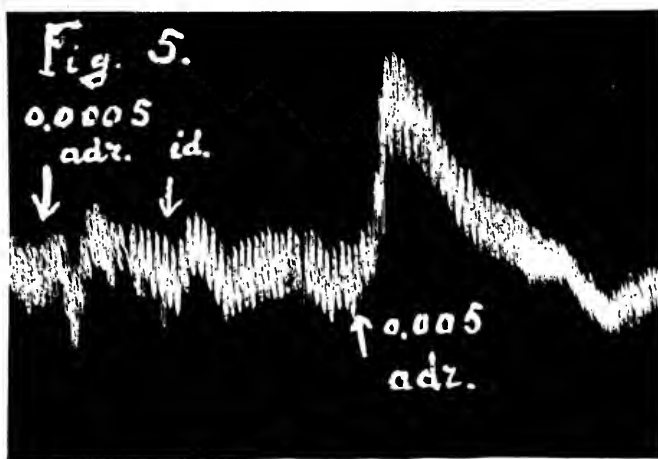
FIG. 5. ACTION OF ADRENALINE ON BLOOD PRESSURE OF CAT SUFFERING FROM EXPERIMENTAL POLYNEURITIS

0.0005 mgm. of adrenaline gives fall in blood pressure; same dose two minutes later hardly any reaction; 0.005 mgm. of adrenaline gives definite rise in blood pressure.

Reaction to adrenaline. The reaction to adrenaline was normal. Cat II gave a drop of blood pressure after injection of 0.0005 mgm. of adrenaline, hardly any reaction after another dose of 0.0005 mgm. and a distinct rise after 0.005 mgm. of adrenaline (fig. 5). The adrenaline (Parke, Davis and Company) was dissolved in 1 cc. of NaCl solution and injected in the vena femoralis at a constant rate, the duration of each injection being exactly twenty seconds.

Cat III gave a distinct rise in blood pressure after 0.0003 mgm. of adrenaline.

These adrenaline doses fall within the range of reactions of a large number of normal animals studied by us in this respect; the minimum active doses of adrenaline varied in these normal cats from 0.0005 to 0.005 mgm.

Reaction to histamine. Ergamine (Burroughs, Welcome and Company) gave a fall of blood pressure in doses of 0.01 and 0.05 mgm. This reaction corresponds completely with that found in
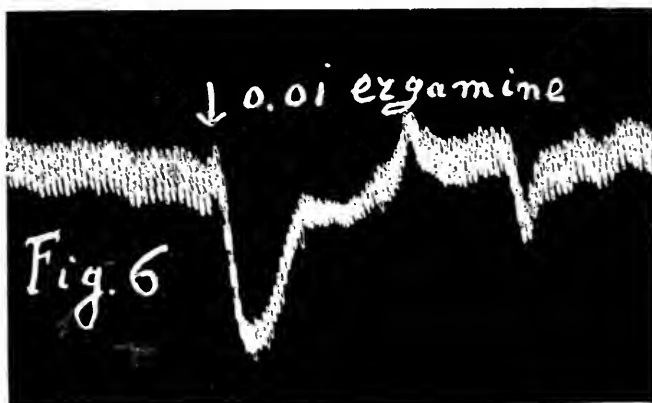
FIG. 6. FALL IN BLOOD PRESSURE CAUSED BY 0.01 MGM. OF ERGAMINE IN A CAT SUFFERING FROM EXPERIMENTAL POLYNEURITIS

normal cats. Figure 6 gives an instance of the action of 0.01 mgm. of ergamine on a cat suffering severely from polyneuritis.

Action of atropine on effect of vagus stimulation. The reaction to atropine was normal in one case, 0.005 mgm. giving a temporary inhibition of vagus effect, lasting for three or four minutes; a second equal dosis then gave the same effect. In a second case 0.0008 mgm. of atropine was active but the effect was doubtful and of very short duration so that we are inclined, especially in view of the results obtained with fowls, to disregard this finding.

Action on isolated gut

Pilocarpine gave normal reactions; doses varying from 0.005 to 0.1 mgm. of pilocarpine hydrochloride added to 75 cc. of Tyrode solution in which the gut was suspended gave a normal contraction which could be inhibited by doses of atropine varying from 0.001 to 0.005 mgm. These doses are exactly equal to those usually found on the gut of normal cats. Figure 7 gives an instance of the action of pilocarpine on the isolated gut of a cat
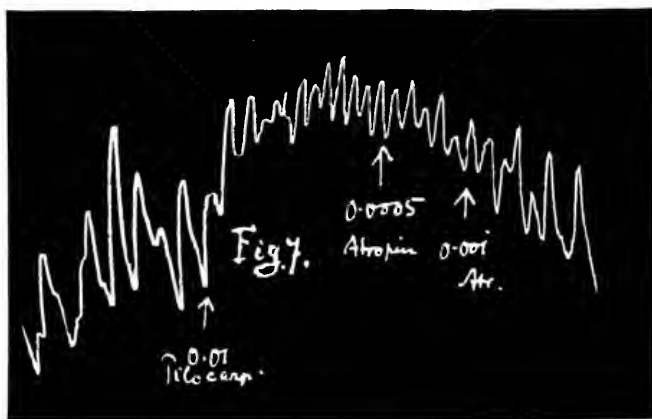
FIG. 7. ACTION OF 0.01 MGM. OF PILOCARPINE ON ISOLATED GUT OF A CAT SUF-
FERING FROM POLYNEURITIS

The pilocarpine action is inhibited by 0.0015 mgm. of atropine

suffering severely from polyneuritis. 0.01 mgm. pilocarpine gives a moderate contraction. 0.0015 mgm. of atropine acted antagonistically and brought back the contraction to the normal niveau.

Ergamine was active in doses varying from 0.02 to 0.05 mgm. and choline in doses of 1 to 2 mgm. These doses also fall in the range of those which cause contractions on normal animals.

DISCUSSION

The object of our investigations was to make out whether a decrease of sensitiveness of smooth musculature to chemical agents normally present in the body could be responsible for

part of the very severe symptoms which occur in animals fed on a deficient diet. We found that the reaction of fowls and cats, suffering from avitaminosis, to adrenaline, histamine and choline (action on blood pressure), to atropine (inhibition of vagus stimulation) and the reaction of isolated gut and esophagus of these animals to pilocarpine, atropine, histamine and choline did not differ materially from the reaction of normal animals or of isolated organs of normal animals.

Since it is quite sure that the function not only of striped musculature, but also of smooth musculature is greatly damaged in animals suffering from avitaminosis and since—as we have shown—the sensitiveness of the smooth musculature to drugs has not been changed it must be deemed very probable that the decreased activity of smooth musculature in avitaminosis is caused by a lack in the body of these animals of the normal stimulating chemical agents. This view is in agreement with the conception of Abderhalden, Bang, Uhlmann and others who ascribe to the vitamins an action very similar to those of pilo-carpine, viz., a stimulating action on smooth musculature.

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THE PHYSIOLOGICAL STANDARDIZATION OF EXTRACT OF BELLADONNA

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It is known that the strength of galenic preparations derived from different sources may vary considerably. Hence it will always be necessary to control the strength of such preparations before allowing them to be used in therapeutics. Very often a chemical standardization may suffice. There are, however, several instances where such a chemical determination either is impossible or gives inaccurate results. Generally speaking this happens in the following cases:

1. Either the active principle is not known or a chemical determination of it is too difficult for routine work, as in the case of digitalis, secale cornutum, tincture of lobelia and others.

2. The drug contains more than one active principle which may or may not be known chemically but there may be reason to believe that some of the constituents of the drug may intensify the action of others ("potentiated" synergism), e.g., opium.

3. The drug may contain—besides the known active principles—other constituents which may inhibit or intensify the action of the active principles.

In all these cases chemical methods of standardization will obviously fail, so that physiological determination becomes necessary.

The active principle of extract of belladonna is known. Often it is stated to contain hyoscyamine and atropine. Schutte (1), however, has shown that wild growing belladonna contains almost exclusively hyoscyamine (only very small quantities of atropine being found), whereas Fiebert, van Itallie (2) and Hagelvoort (3), found that the same holds true for cultivated belladonna and for extract of belladonna.

313

Extract of belladonna then contains only one active principle and a chemical determination of this principle is possible. The Dutch pharmacopaea gives a method for the determination of the total amount of alkaloids present in extract of belladonna and requires that this should not be below 1.15 per cent. Apart from the fact that it might appear unwise to require only a minimum instead of requiring also a maximum, as the United States pharmacopaea does, for the percentage of alkaloids in a drug, this state of affairs seemed to be satisfactory. From time to time, however, clinicians complain that certain preparations of belladonna though complying with the requirements of the pharmacopaea have a weaker action than other preparations. Belladonna is considered by many clinicians to be a drug of inconstant strength.

Notwithstanding the fact that belladonna seems to contain only one active principle, the amount of which can be determined chemically, we deemed it possible that the doubt of the clinicians as to the constancy of strength of belladonna might be justified by the occurrence of unknown constituents in this drug which might increase or inhibit the action of belladonna.

We therefore procured samples of belladonna-extract from six different apothecaries in the country and determined the strength by means of physiological methods. After that we sent a portion of the same samples to Dr. Meulenhof in Zwolle who kindly made chemical determinations for us following exactly the prescriptions of the Dutch pharmacopeae.

In looking for a method of physiological standardization of belladonna we were led by the following considerations.

If it is true that belladonna preparations of the same strength (according to chemical investigations) are not always of equal use in therapeutics, it must be deemed possible after all that some preparations contain considerable amounts of atropine so that chemical determination of the total amount of alkaloids would give very inaccurate results.

The physiological action of atropine and of hyoscyamine on various organs in the animal body has been carefully studied by

Cushny.   His results as far as they are to be considered here are summarized as follows (4):

Pure atropine and hyocyamine act in the same way and with equal potency on the central nervous system in mammals and on the heart and terminations of the motor nerves in the frog.   Atropine possesses a more powerful (stimulant) action on the reflexes of the spinal cord of the frog than hyoscyamine.   Hyoscyamine is almost twice as powerful as atropine in its action on the nerve ends in the salivary glands, heart and pupils.

It is clear then that the best way to study the action of various belladonna preparations would be to determine their action on the salivary glands and on the heart and the pupils.   We chose the action on the salivary glands and followed exactly the method described by Cushny (5), which after a few preliminary determinations proved to give excellent results.

We used two dogs, each of which had a permanent fistula of one of the submaxillary ducts, made some weeks before the first research was made.   Each dog was only used twice a week.   After having found the amount of atropine which in each dog was necessary to inhibit the increased secretion of saliva produced by the injection of pilocarpine, we injected various quantities of each sample of extract of belladonna until (vide Cushny, loc. cit., p. 106) it was found that a quantity (x) of the belladonna solution was weaker than (y) of atropine, while $x + a$ of hyoscyamine was stronger than y of atropine.   Several determinations were made with each sample of belladonna and in the end the average values were taken.   As it was to be expected from the statement made above that the extract of belladonna would as a rule contain only hyoscyamine and as the action of hyoscyamine on the salivary gland is twice as powerful as the action of atropine we had of course to divide our values by 2 in order to be able to compare them with the results of the chemical determinations.

The values found for the different preparations of extract of belladonna are given below in tabular form:

SAMPLE	PERCENTAGE OF HYOSCYAMINE PHYSIOLOGICAL DETERMINATION	PERCENTAGE OF TOTAL ALKALOIDS CHEMICAL DETERMINATION
	per cent	per cent
A	1.875	1.56
B	1.4	1–41
C	1.76	0.65
D	1.445	0.95
E	1.24	0.81
F	0.85	0.995

It will be seen from this table, that in some instances the results of the physiological and the chemical determinations agree fairly well (samples A, B and F) in two instances there are rather remarkable differences (E, D), whereas in sample C there is no agreement at all between the values obtained by the two methods. We have stated above that we thought it possible that—notwithstanding the results of former investigations on this point—some samples of belladonna might contain atropine. This however could never explain the difference in C, E and D as the occurrence of atropine in these samples would have given a deviation in the other direction, viz., the physiologically determined values would have been too low instead of too high.

Before trying to find an explanation for the fact that so little agreement existed between the results of the two methods we wanted to exclude errors and therefore we ordered another sample of belladonna from the same pharmacy and had new chemical and physiological determinations made thereon; they gave exactly the same results. The fact that the physiological method gave in the case of sample C (and also in E and D) so much higher values than the chemical method can according to our view be explained in one of two ways:

a. Either the extract of belladonna C contains—besides a certain amount of hyoscyamine—a quantity of an alkaloid which has a more powerful action on salivary secretion than hyoscyamine.

b. Or the belladonna contains substances which increase the action (or promote the resorption after subcutaneous injection) of hyoscyamine.

The first assumption seems to be improbable since no alkaloids of a stronger action on salivation than hyoscyamine are known.

However to exclude this possibility we removed by extraction with ether (following the prescription of the pharmacopaea) the total alkaloids from sample C and determined the strength thereof with our physiological method. The value found agreed—within the range of experimental error—with the value of the chemical determination so that it may be considered highly improbable that sample C contained unknown alkaloids with high degree of action on salivary secretion. Very likely then the second assumption will be true, i.e., sample C will have contained substances which increase the action of pilocarpine or improve its resorption after subcutaneous administration of the drug. It was not possible to differentiate between these two alternatives since no further quantities of this brand were available. Information as to the nature of this promoting substance can—for the same reason—not yet be given. The only fact known is that it does not dissolve in ether.

It may be of interest in this connection to mention a recent communication of Kunz-Krause (6) who in determining the strength of belladonna extracts with chemical and physiological methods (mydriatic action on the eye of rabbit and cat) found a difference in the opposite direction from our case, the chemical determination giving a value nine times as high as the physiological method. He thinks that his extract of belladonna must have contained colloidal substances which could inhibit the action of the alkaloid present.

Before it is possible to draw definite conclusions from the experiments communicated above it would be necessary to know whether substances in extract of belladonna which can increase or inhibit the action of hyoscyamine can exert that same action in the human body. In our researches we injected the drug subcutaneously in dogs, but in human practice it is mostly given per os, and we do not know whether in the alimentary canal the drug promoting or drug inhibiting, influences described will still be able to play a rôle. We are of the opinion that it is highly probable that such is the case, but it will be very difficult to investigate this matter experimentally. We think, however, that as long as it has not been proved that these substances have no

influence in the alimentary canal it is safe to assume that they have one.

In a case where—as in sample C— the physiological method gives a much higher value than the chemical one, the question arises which value has to be considered as an indication of the real strength of the preparation. We incline to the view that the physiological determinations have to be decisive but we think that as long as this has not been proved it will be advisable to reject those preparations which show too wide a divergence between both these methods, or anyhow to exclude such preparations from use in important cases.

In all cases where accurate prescriptions are wanted we advise recourse to a standardized preparation and we propose to consider as "standardized extracts of belladonna" a preparation containing exactly 1.15 per cent of alkaloids for Holland or 1.18 to 1.32 per cent for the United States (determined by means of the usual chemical method) and which gives—when standardized physiologically—a value not differing materially from 1.15 per cent (1.18 to 1.32 per cent) of hyoscyamine.

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THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE TOXICITY OF ALKALOIDS FOR PARAMOECIUM

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The explanation of the antagonistic action of certain alkaloids (e.g., atropine and pilocarpine) as being on nerve endings has been questioned by some investigators who suggest that the action is on the cell itself. In an attempt to test whether the typical antagonism could be demonstrated on a unicellular organism, the effect of slight changes in the hydrogen ion concentration of the solutions in influencing the toxicity presented a difficulty. Although the effect of acid and alkali upon the toxicity of substances and upon their penetration into the living cell is well known, no quantitative data could be found as to the extent of change in hydrogen ion concentration necessary to produce a certain effect. Some of this kind were found for bacteria and are mentioned below. The present work was undertaken to study the effect of known changes in hydrogen ion concentration on the toxicity of certain alkaloids and bases, and presents data which lends support to the idea that the change in toxicity is due to an effect on the drug and not on the cell.

The questions of cell penetration and toxicity are so intermixed that they must be discussed together. It must be borne in mind however that the relations are not entirely the same, as is seen from the results of Harvey (1911, 1914) who found that strong acids and alkalies kill without penetrating the cell. On the other hand he concluded that the toxicity of weak acids and bases depends upon ability to penetrate the cell.

With a few exceptions previous investigators have found that

319

salts of weak bases penetrate the cell more rapidly and are more toxic in alkaline than in acid solution, while acid substances penetrate less readily from alkaline solution. This has been held to support the theory proposed by Overton (1896) that the penetration of the salts of weak bases is dependent upon tne hydrolytic splitting of the compound. The effect of acid or alkali upon the penetration or toxicity of drugs is therefore considered to be entirely due to an effect on the drug.

Other investigators believe that the variation in toxicity with change in hydrogen ion concentration is due to an action directly upon the cell. The work of Osterhout (1914) in particular supports this idea for the penetration of inorganic salts into Laminaria.

Previous investigations of the effect of changes in reaction upon the toxicity or penetration of compounds have been made by adding small quantities of acid or alkali to the solution to be tested, or by comparing the action of equimolecular solutions of salt and pure alkaloidal base. The actual variation in hydrogen ion concentration has been measured only in a few isolated observations on bacteria and here no attempt is made to connect the results with previous work on the subject. The difficulty in obtaining definite or constant hydrogen ion concentrations by merely adding acid or alkali is well known and it is impossible to compare the results of the experiments in more than a very general way without the knowledge of the variations in hydrogen ion concentration involved.

Overton's (1896, 1899) theory of the dependence of penetration of the salts of weak bases upon hydrolysis was suggested to him by the observation that salts of the base with weak acids penetrate Spirogyra cells more rapidly than salts with strong acids. He found confirmation for the idea in the fact that addition of acid to the salt solution, depressing hydrolysis, prevented any appreciable penetration of the base into the cell. The relations for basic dyes were the same. A study of the toxicity of certain alkaloids for plant cells also supported the theory. He found the salts of the alkaloids much less toxic than the free alkaloid, and the addition of a slight amount of free acid caused a marked decrease in toxicity.

Robertson (1908) studied the penetration of both acid and basic dyes into fat cells, connective tissue cells, and red blood corpuscles. He concluded that basic dyes penetrate better in alkaline solution and acid dyes better in acid solution. His experiments have been criticized by Harvey (1911) who believes that his acid and alkali solutions were too strong for the cells to have been alive.

Loeb (1898) found atropine to be less toxic for Paramoecium if in alkaline solution. Strychnine and veratrine were not affected by a change in reaction. Prowazek (1910), on the other hand, found alkaline solutions of strychnine, atropine and quinine and of certain basic dyes to be more toxic for Colpidia than acid solutions. Similar results were obtained by Traube (1912) in determining the toxicity of these alkaloids for tadpoles. Přibaim (1911) found that the haemolytic action of cocaine on red blood cells is greater in alkaline solution.

In 1911 Harvey made a very careful study of the penetration of aniline dyes into living cells. He used Spirogyra and the yolk platelets of the frog's egg as representative of plant and animal cells. His results indicate that in acid solution ($\frac{N}{1000}$ HCl) only the acid dye penetrates, while in solutions containing $\frac{N}{1000}$ NaOH only basic dyes can enter the cell. He believes with Overton that the action of the acid is on the penetrating substance rather than on the cell, because the relations for basic dyes are reversed for acid dyes, and because the addition of acid to solutions of the heavy metals does not affect their toxicity. In comparing his results with those obtained by Overton for alkaloids, Harvey points out that caffeine, unlike most alkaloids, penetrates equally well in acid, neutral and alkaline solution, but he does not discuss the reason for this difference. Endler (1912) investigated the effect of acids and alkalies on the permeability of a number of different plant cells to dyes. He found that OH-ions in low concentrations increase the diffusion of the dye either into or out of the cell, but that higher concentrations have an inhibitory action. H-ions he found decreased the basic dye entering the cell but increased the penetration of the acid dye. Amphoteric dyes penetrated equally well in acid or alkaline solution. Tröndle (1920) recently observed that quinine penetrates the plant cell more slowly from solutions of its salts than from solutions of the free base, and still more slowly from solutions of the salt to which acid has been added.

The relative activity of the free base and of the salts of cocaine and its derivatives in blocking nerve was determined by Gros (1910). He found the free base to be much more efficient. His solutions were

mixtures of the hydrochloride of the base, sodium chloride, and, when the free base was desired, slightly less than the calculated amount of NaOH. Symes and Veley (1911) however were unable to confirm these results when using solutions of the pure alkaloidal base and of the salt.

In their studies of urinary antiseptics, Davis, White and Rosen (1918) found chlor-mercury fluorescein to be more efficient as an antiseptic in alkaline than in acid urine. Acriflavine and proflavine they found to be more toxic for B. coli in alkaline urine, but equally toxic for Staphylococcus in either acid or alkaline urine. The authors offer no explanation of the effect of change in reaction on the antiseptic properties of these drugs. Such a difference in the action on B.coli and on Staphylococcus would seem to indicate some effect on the cell rather than on the drug.

Graham-Smith (1918) determined the antiseptic action of homoflavine and quinone at different hydrogen ion concentrations, using B. coli, Staphylococcus and B. pyocyaneus. He found homoflavine more effective in alkaline solution and quinone more effective in acid. The curve of the quinone determinations is so irregular however, as to be somewhat unconvincing. Browning, Gulbransen and Kennaway (1919) found a decrease in the toxicity of certain acridine compounds for *B. coli* with increasing hydrogen ion concentration.

These determinations of the effect of hydrogen ion concentration upon antiseptic action are the only investigations of this nature which I have found in which the pH is measured, and here the authors have not connected their observations with any of the other work on the subject or attempted in any way to discuss the theoretical interpretation of their results.

The very careful studies of the relative permeability of the cell to different acids and alkalies which have been made by Barratt (1904) Loeb (1909), Harvey (1911, 1914), Crozier (1916) and Haas (1916), do not immediately bear upon the present problem.

While most of the work quoted above supports the idea of an effect of the hydrogen or hydroxyl ions upon the drug, the results of Osterhout (1914) seem to indicate an action directly upon the cell. He studied the effects of acid and alkali on permeability for salts by measuring the changes in electrical conductivity caused in Laminaria saccharina by the addition of NaOH or HCl to the sea-water or NaCl-CaCl₂ solutions used as control media. The addition of small amounts of NaOH caused a fall in resistance indicating an increased permeability

of the cell.   HCl on the other hand caused at first a rapid decrease in permeability which was followed immediately by a more gradual increase until the death of the cell.   The H- and OH-ions have evidently some direct effect on the permeability of the cell to inorganic salts.

As a whole, the previous work seems to show that the salts of many organic bases penetrate the cell more easily and are more toxic in alkaline than in acid solution, although the results of Loeb (1898) and certain observations on bacteria do not show this effect.   The magnitude of the variation with definite changes in hydrogen ion concentration has apparently not been investigated however except in a few isolated determinations on bacteria.   The observations in most cases support the idea that thanges in hydrogen ion concentration affect toxicity through their effect on the drug, but the possibility of an action directly upon the cell still remains.   The present experiments determine the extent to which definite changes in hydrogen ion concentration affect the toxicity of various bases, and offer additional evidence that the effect is due to action on the drug and not on the cell.

EXPERIMENTAL

Method.   The effect of changes in hydrogen ion concentration on the toxicity of certain alkaloids and basic compounds was studied on Paramoecium caudatum.   The strain used was developed from a single organism isolated from a culture very kindly furnished by Dr. Grave of the Washington University Department of Zoölogy.   The Paramoecia was grown in a culture media made by boiling timothy hay in distilled water. The infusion was filtered and a few pieces of the boiled hay added. The culture was allowed to stand at room temperature.   When used the cultures were from four to ten or occasionally fourteen days old.

The experiments were carried out in small homepathic vials. Into each vial was pipetted the necessary amount of the solution of the drug and 0.2 cc. of an $\frac{M}{15}$ sodium phosphate buffer mixture. Sufficient water was added to bring the total volume, after

addition of the Paramoecium suspension, to 2 cc. The solution of the drug was neutralized before being added so as to cause very little change in the reaction of the mixture. The Paramoecia were added last, as rapidly as possible so that all the tubes in an experiment were started at almost exactly the same time. In most of the determinations the culture was centrifuged at low speed for a few minutes in order to give a heavier suspension of the organism. The amount of the culture added varied from 0.1 to 0.3 cc., but was uniform throughout each experiment. There were usually fifty or more Paramoecia in each tube. Owing to the well known variations in the resistance of Paramoecium to ·drugs with differences in age, media, light, etc., only those results are compared which were obtained at the same time and under exactly the same conditions.

At the end of the experiment the exact hydrogen ion concentration of the mixture was determined by the addition of a suitable indicator and comparison with standard buffer solutions. The indicators used were those recommended by Lubs and Clark (1915).

In order to control the hydrogen ion concentration of the solutions used in the experiments, a sodium phosphate buffer mixture was added. The lowest concentration of the buffer which could be depended upon to keep the reaction constant was found to be $\frac{M}{150}$. In this concentration the salt showed no toxic action on Paramoecium, and control tubes lived indefinitely. The lethal concentration of the phosphate is approximately $\frac{M}{35}$.

Observations were made with the naked eye. The time was recorded at which most of the Paramoecia in a tube ceased to swim and sank to the bottom. Owing to the variations in the resistance of individuals it was occasionally difficult to decide exactly what to call the time of death, but in most cases it was fairly definite and such inaccuracies are relatively small. Two, or more often three tubes of each mixture were run simultaneously, and the agreement was usually very close in identical solu-

tions. After an experiment was started the tubes were observed continually for one or two hours, and after that usually about once every hour for several hours. A final observation was made after twenty-four hours. It was found in most cases Paramoecia lived indefinitely in a solution in which they survived more than twenty-four hours.

Determination of toxicity of H- and OH-ions. Before testing the effect of hydrogen ion concentration on other substances it was necessary to determine the toxicity of the hydrogen and hydroxyl ions themselves for Paramoecium. Barrett (1904) and Harvey (1911) studied the toxicity of acids and alkalies, but not of accurately controlled hydrogen and hydroxyl ion concentrations. Dorothy Dale (1913) determined the pH limits within which Paramoecium can live in various buffer solutions. She does not give any figures however for periods longer than one hour and for the purposes of the present experiments it is necessary to know the H^+ and OH^- concentrations which kill within twenty-four hours.

Miss Dale found the limits of hydrogen ion concentration within which Paramoecium can live 100 seconds in a solution buffered with sodium acetate-acetic acid to be $1 \times 10^{-5.52}$ and $1 \times 10^{-11.53}$. In solutions of glycocoll-HCl and glycocoll-NaOH the limits were $1 \times 10^{-4.05}$ and $1 \times 10^{-11.68}$, and in sodium citrate-HCl and sodium citrate-NaOH solutions her figures are $1 \times 10^{-3.91}$ and $1 \times 10^{-10.5}$. With $\frac{M}{20}$ acetate buffer pH of 5.8, killed in twenty-five minutes; with $\frac{M}{60}$ sodium citrate-NaOH buffer, 9.5 killed in sixty minutes.

Miss Dale used buffer solutions in final concentrations of $\frac{M}{60}$ to $\frac{M}{20}$. These concentrations were not lethal at hydrogen ion concentrations which had no action upon the cell. But as the H- or OH-ion concentration increases, the toxic action of the salts is added to that of the H- and OH- ions and the death of the cell would be due to their combined action. In solutions

containing so much buffer there is undoubtedly some action of the salt ions and the figures for lethal concentrations of H- and OH-ions obtained in this way are unquestionably inaccurate. Since we cannot add hydrogen and hydroxyl ions alone, it is impossible to avoid having some other ions present which may affect the cell, so any figures on the toxicity of H- and OH-ions will be higher than the actual values. By using the most dilute solutions of buffer which will control the reaction and by comparing the results obtained from different buffer mixtures, approximate figures can be obtained which will be somewhat smaller than the actual maximum concentrations of H- and OH-ions compatible with life.

The toxicity of the H-ions for Paramoecium was determined in three buffer mixtures: Glycocoll-hydrochloric acid, acid potassium phthalate-sodium hydroxide, and a mixture of monobasic and dibasic sodium phosphate. The sodium acetate-acetic acid buffer was found to be too toxic even in very dilute solutions, giving values very different from those obtained with other buffers. In a sodium phosphate solution containing $\frac{M}{100}$ phosphate, Paramoecia lived twenty-four hours at pH 5.2, but died within twenty-four hours at pH 5.1. In a phthalate-NaOH buffer, containing $\frac{M}{1000}$ phthalate, they lived at 5.0 but died at pH 4.8. The figures obtained with a glycocoll-HCl mixture in a concentration of $\frac{M}{400}$ glycocoll are the same as those obtained with the phthalate buffer. The hydrogen ion evidently becomes toxic for Paramoecium in a concentration of about $1 \times 10^{-5.0}$.

The lethal concentration of OH-ions was determined with only one buffer solution. In a mixture of glycocoll and NaOH containing $\frac{M}{400}$ glycocoll, the organisms died in less than twenty-four hours at pH 9.7, but lived in a solution of pH 9.6. The most alkaline solutions obtainable with dilute sodium phosphate do not kill. The maximum hydroxyl ion concentration within

which Paramoecium can live may be considered as approximately pH 9.6.

Effect of changes in H-ion concentration on toxicity. Using the method described above, determinations were made of the toxicity of atropine, strychnine and quinine in solutions at various hydrogen ion concentrations. The results of only a few experiments can be presented here in full, and the more significant figures from other typical experiments are reported. Figures typical of the results obtained with strychnine and quinine are presented in tables 1 and 2. In these tables, as in others following, the figures represent the time at which death occurred, measuring from the time at which the Paramoecia were added to the solution of the drug. Two, or in most cases, three or more separate series of determinations were made for each drug on different days, so that the experiments reported have in every case been repeated at least once with similar results. Figures are given of the time of death of duplicate or triplicate tubes run at the same time.

The figures obtained from experiments with atropine show the same relations as those obtained with strychnine and quinine. 0.60 per cent solutions of atropine sulphate killed immediately at pH 8.0; in 1 minute at pH 7.0 and in fifty minutes at pH 6.4. The lethal concentrations in 24 hours were: 0.10 per cent at pH 8.0; 0.20 per cent at pH 7.0; and 0.40 per cent at pH 6.4.

The results of the experiments indicate very definitely that the alkaline solutions of these alkaloids are more toxic for Paramoecium than the acid solutions. In order to ascertain that the differences in toxicity are not due to an action of the sodium phosphate, determinations similar to those just presented were made in which the hydrogen ion concentration was controlled by the addition of sodium carbonate, and others in which solutions of quinine base and salt were used without the addition of buffer. The figures obtained resemble very closely those obtained with the sodium phosphate buffer. Another experiment was run in which two complete series of determinations were made, using twice as much buffer in one series as in the other. If the differ-

TABLE 1

Effect of variation in H-ion concentration on the toxicity of strychnine

FINAL CONCENTRATION OF STRYCHNINE NITRATE	pH 8.7	pH 8.0	pH 7.0	pH 6.4	pH 5.9
per cent	minutes	minutes	minutes	minutes	minutes
0.220 {	I I	I I	I I	I I	28 28
0.200 {	I I	I I	I I	I I	55 55
0.180 {	I I	I I	I I	I I	70 70
0.160 {	I I	I I	I I	I I	55 55
0.150 {	I I	I I	I I	30 30	135 135
0.140 {	I I	I I	I I	55 55	100 130
0.120 {	I I	I I	I I	60 60	240 240
0.100 {	I I	I I	3 3	210 210	24 hours 24 hours
0.080 {	I I	I I	5 17	24 hours 24 hours	L L
0.060 {	I I	2 2	60 60	24 hours 24 hours	L L
0.040 {	2 2	2 3	150 150	24 hours 24 hours	L L
0.020 {	85 85	105 105	210 210	L L	L L
0.010 {	150 150	24 hours 24 hours	24 hours 24 hours	L L	L L
0.008 {	210 210	24 hours 24 hours	L L	L L	L L
0.004 {	24 hours 24 hours	24 hours L	L L	L L	L L
0.002 {	L L	L L	L L	L L	L L

I indicates Paramoecia were dead before tube could be examined.
L indicates Paramoecia were alive after twenty-four hours.

TABLE 2

Effect of variations in H-ion concentration on the toxicity of quinine

FINAL CONCENTRATION OF QUININE SULPHATE	pH 8.0	pH 7.4	pH 7.0	pH 6.4	pH 5.9
per cent	minutes	minutes	minutes	minutes	minutes
0.0200	0.5	1	2	5	11
	0.5	1	2	5	12
	0.5	1	2	15	
0.0100	1.5	4	7	21	75
	1.5	4	7	21	85
	1.5	4	7	21	
0.0050	3	9	23	330	270
	3	9	23	330	270
	3	10	31	330	
0.0030	9	26	90	L	L
	9	26	90	L	L
	9	26	90	L	L
0.0025	16	34	330	L	L
	19	46	300	L	L
	15	46	140	L	L
0.0020	25	125	L	L	L
	25	125	L	L	L
	27	L	L	L	L
0.0015	29	240	L	L	L
	67	L	L	L	L
	L	L	L	L	L
0.0014	72	L	L	L	L
	100	L	L	L	L
	100	L	L	L	L
0.0010	200	L	L	L	L
	200	L	L	L	L
	L	L	L	L	L
0.0008	L	L	L	L	L
	L	L	L	L	L
	L	L	L	L	L

L indicates that Paramoecia were alive after twenty-four hours.

ences in toxicity were due to the buffer salts, they should be greater in the series containing more buffer. Such was not found to be the case, there was very close agreement between the two series.

We see from these experiments, therefore, that a decreased hydrogen ion concentration increases the toxicity of solutions of strychnine, atropine and quinine. The increase may be explained as due to an effect of the hydrogen or hydroxyl ions upon the drug, or an action directly upon the cell.

If the action is upon the cell we should expect the effect of a certain change in hydrogen ion concentration to be more or less the same on the toxicity of all compounds in which differences in physical characteristics are not too great. In order to test this idea, determinations were made of the effects of known changes in reaction on the toxicity of certain neutral substances; chloroform, acetone and oil of peppermint.

Solutions of 0.1 per cent chloroform killed at pH 8.0 in 8, 8 and 8 minutes; at pH 7.2 in 10, 12 and 12 minutes; at pH 6.4 in 12, 12 and 12 minutes. The figures represent the results obtained in triplicate tubes. 0.050 per cent chloroform at pH 8.0 killed in 150, 210 and 240 minutes; at pH 7.2 in 210 minutes and less than 24 hours, while the third tube lived twenty-four hours; at pH 6.4 one tube died in 210 minutes and the other two tubes lived. In 0.025 per cent chloroform Paramoecia lived twenty-four hours at all hydrogen ion concentrations studied.

In a number of determinations the toxicity of oil of peppermint was practically unchanged by differences in hydrogen ion concentration. The lethal concentration of acetone in twenty-four hours was 2 per cent at pH 7.8 and 3 per cent at pH 6.0. Evidently there is very little effect from hydrogen ion concentration upon the toxicity of these neutral substances. Changes in hydrogen ion concentration apparently have an influence on the toxicity of atropine, strychnine and quinine which is not found in the case of neutral substances.

A determination of the variations in toxicity of caffeine citrate in solutions of different hydrogen ion concentration placed caffeine in the class with the neutral substances rather than with the other alkaloids studied (see table 3). This seemed

rather surprising at first. If the action of hydrogen ions is directly upon the cell, we should expect the resistance to all alkaloids to be altered to more or less the same extent. If the effect is upon the drug, differences in hydrogen ion concentration will affect the hydrolysis of all salts of weak bases. The magnitude of the effect upon hydrolysis will vary however with the strength of the base. A comparison of the dissociation constants of the bases studied revealed a striking variation in this factor. (The values for the dissociation constants used are those given by Scudder (1914). They were determined at temperatures vary-

Dissociation constant of base

Atropine	1.0×10^{-7}
Quinine	17.0×10^{-7}
Strychnine	8.6×10^{-7}
Caffeine	1.0×10^{-10}

TABLE 3

Effect of variations in H-ion concentration on the toxicity of caffeine

FINAL CONCENTRATION OF CAFFEINE CITRATE	pH 8.0			pH 7.2			pH 6.4		
per cent	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes
0.8	4	4	4	4	4	4	4	4	4
0.6	10	10	10	10	10	10	11	11	11
0.5	11	11	11	13	12	12	13	22	25
0.4	27	27	42	27	27	37	75	75	75
0.3	55	75	70	105	105	105	150	105	150
0.2	105	180	180	165	165	195	210	210	210
0.1	330	330	330	315	300	300	315	315	315
0.05	L	L	L	L	L	L	L	L	L

L indicates that Paramoecia were alive after twenty-four hours.

ing from 15° to 25°C., but the errors due to variations in temperature are relatively small.)

While the constants for atropine, strychnine and quinine are all of the same order of magnitude, that for caffein is much smaller. This seemed to indicate a definite relationship between the dissociation constant of the base and the effect of variations in hydrogen ion concentration on the toxicity of solutions of its salts.

With this possibility in mind, determinations were made of the variations in toxicity with changing hydrogen ion concentration of the solutions of two groups of basic compounds, one group having very small dissociation constants, and the other group having much larger constants. In the first group are a-naphthyl amine, p-phenetidine, and quinoline, in the second group d-coniine, piperidine and amylamine were tested. Results typical of each group are presented in tables 4 and 5.

TABLE 4

Effect of variations in H-ion concentration on the toxicity of naphthyl amine

FINAL CONCENTRA-TION OF A-NAPHTHYL AMINE HYDROCHLORIDE	pH 8.0		pH 5.9	
per cent	minutes	minutes	minutes	minutes
0.0400	3	3	3	6
0.0350	4	4	5	6
0.0300	9	9	10	11
0.0250	11	16	17	17
0.0200	15	17	21	21
0.0150	30	30	35	35
0.0100	40	40	45	50
0.0050	105	105	105	105
0.0025	24 hours	24 hours	24 hours	24 hours
0.0015	24 hours	24 hours	L	L
0.0007	L	L	L	L

L indicates that Paramoecia were alive after twenty-four hours.

TABLE 5

Effect of variation in H-ion concentration on the toxicity of piperidine

FINAL CONCENTRA-TION OF PIPERIDINE HYDROCHLORIDE	pH 8.0		pH 5.9	
per cent	minutes	minutes	minutes	minutes
2.00	3	3	8	8
1.60	4	8	13	13
1.20	9	9	24 hours	24 hours
0.80	10	10	L	L
0.40	15	15	L	L
0.20	22	22	L	L
0.10	24 hours	24 hours	L	L
0.05	24 hours	24 hours	L	L
0.025	L	L	L	L

L indicates that Paramoecia were alive after twenty-four hours.

The concentration of phenetidine hydrochloride which killed in two minutes was 0.40 per cent, both at pH 8.0 and pH 6.0. The concentration which killed in three hours was 0.25 per cent at pH 8.0, and 0.30 per cent at pH 6.0. The twenty-four hour lethal concentration was 0.5 per cent at both hydrogen ion concentrations. 0.060 per cent quinoline hydrochloride killed in one hour at pH 8.0; 0.075 per cent at pH 5.9. In four hours 0.04 per cent killed at pH 8.0; 0.05 per cent at pH 5.9. The twenty-four hour lethal concentrations were 0.030 per cent and 0.035 per cent respectively.

In the second group, a 0.03 per cent solution of amylamine hydrochloride killed at pH 8.0, while it took 0.35 per cent to kill at pH 6.0. Coniine killed immediately in 0.36 per cent solution at pH 8.0, but not for twenty minutes in 0.48 per cent solution at pH 6.0. Twenty four hour lethal concentrations were 0.0075 per cent at pH 8.0 and 0.1200 per cent at pH 6.0.

On examination of these results it is evident that there is a marked effect from variations in hydrogen ion concentration in the case of the bases having comparatively large dissociation constants, but little or no effects on the bases with very small constants. This relationship is more clearly brought out in table 6, in which the magnitude of the variation in toxicity with definite changes in reaction is presented in conjunction with the values for the dissociation constants. In the first column is given the name of the drug, in the second column the pH at which its toxicity was determined. The third column gives the concentration which kills in 24 hours at the given pH. In the fourth column is the ratio of the twenty-four hour lethal concentration at a pH of between 5.9 and 6.4 divided by the twenty-four hour lethal concentration at pH 8.0 The last column gives the dissociation constant of the base.

We have here three groups of bases: The caffeine group with very small dissociation constants, the coniine group with much larger constants and the atropine group occupying an intermediate position. There seem to be very definite toxicity relations for the different groups. Changes in hydrogen ion concentration affect the first group very little or not at all, while in the atropine group there is a very definite effect. The dif-

TABLE 6

Summary of effect of variation in H-ion concentration on the toxicity of certain drugs and comparison with their dissociation constants

DRUG	pH	LETHAL CONCENTRATION, 24 HOURS	RATIO	DISSOCIATION CONSTANT
		per cent		
Caffeine	6.4	0.1		
	8.0	0.1	1.0	1.0×10^{-10}
Phenetidine	6.0	0.05		
	8.0	0.05	1.0	2.15×10^{-10}
a-Naphthyl amine	5.9	0.0025		
	8.0	0.0015	1.6	0.99×10^{-10}
Quinoline	5.9	0.035		
	8.0	0.025	1.4	0.8×10^{-10}
Atropine	6.4	0.4		
	8.0	0.1	4.0	1.0×10^{-7}
Quinine	5.9	0.005		
	8.0	0.001	5.0	17.0×10^{-7}
Strychnine	5.9	0.100		
	8.0	0.004	25.0	8.6×10^{-7}
d-Coniine	6.0	0.0600		
	8.0	0.0075	8.0	1.32×10^{-3}
Piperidine	5.9	1.20		
	8.0	0.05	24.0	1.15×10^{-3}
Amyl amine	6.0	0.35		
	8.0	0.03	12.0	5.0×10^{-4}
Chloroform	6.4	0.075		
	8.0	0.050	1.5	
Oil of peppermint	5.9	25.0		
	8.0	30.0	0.8	
Acetone	6.0	2.0		
	8.0	1.0	2.0	

ference between the atropine and coniine groups is less marked, but in general there seems to be a greater increase in toxicity with decreased hydrogen ion concentration in the group with the larger dissociation constants. That these are the results to be expected, if as Overton suggested, the drug acts upon the cell only in the form of the undissociated free base, is to be seen from a consideration of the equilibria involved in such solutions as have been used.

<div align="center">DISCUSSION</div>

If a basic substance affects a cell only in the form of the un-dissociated free base, the relative toxicity of different solutions of the compound must depend upon the proportions of bsae present in this form. In solutions of the salt of a weak base we have theoretically a mixture of the base, BOH, and its salt, BA, although one form or the other may be so depressed as to be practically negligible. The extent to which each form will be present depends upon the dissociation constant of the base, and upon the H-ion concentration of the solution. If we have a base, BOH, with dissociation constant Kb, then according to the mass law we have

$$(B^+) \times (OH^-) = Kb\ (BOH),$$

or the concentration of dissociated base multiplied by the concentration of hydroxyl ions is equal to the concentration of undissociated base multiplied by a constant. For the present purposes this is more conveniently expressed as

$$\frac{(OH^-)}{Kb} = \frac{(BOH)}{(B^+)}$$

When in solution with its salts, the concentration of undis-sociated molecules (BOH) of a weak base is approximately equal to the total concentration of free base, and the concentration of the cations (B^+), is equal to the total quantity of the salt (BA), multiplied by the degree of ionization, γ. Therefore

$$\frac{(OH^-)}{Kb} = \frac{(BOH)}{\gamma\ (BA)}$$

In very dilute solutions very little error is involved in assuming that $\gamma = 1$, and in the present calculations the completeness of ionization affects the concentration of free base to a relatively slight extent and so may be disregarded. The equation therefore becomes

$$\frac{(BOH)}{(BA)} = \frac{(OH^-)}{Kb}$$

Since,

$$(OH^-) = \frac{1 \times 10^{-14}}{(H^+)}$$

the equation may be expressed,

$$\frac{(BOH)}{(BA)} = \frac{1 \times 10^{-14}}{Kb \cdot (H^+)}$$

If we know the dissociation constant of the base and the pH of the solution we can calculate from the above equation the proportion of base present as BOH.

To determine the proportion of free undissociated base in a solution of quinoline hydrochloride at pH 6.0, we have the equation:

$$\frac{(BOH)}{(BA)} = \frac{1 \times 10^{-14}}{0.8 \times 10^{-10} \cdot 1 \times 10^{-6}}$$

whence

$$\frac{(BOH)}{(BA)} = \frac{100}{0.8}.$$

BOH is therefore 99.2 per cent of the total base present in the solution either as free base or as salt. At pH 8.0, 99.9 per cent of the base is in the form of BOH. The difference in proportion of free undissociated quinoline base at pH 6.0 and at pH 8.0 is evidently so slight as to have little if any appreciable effect on toxicity. The relations for strychnine are quite different, the concentration of BOH increasing from 11.5 per cent to 53.7 per cent with a change in pH from 6.0 to 8.0, so that the variation is sufficient to definitely affect toxicity. In the case of piperidine, with a dissociation constant of 1.15×10^{-3} the increase is from 0.0008

per cent to 0.086 per cent. Although the absolute quantity of free undissociated base in the solution is very small at both hydrogen ion concentrations, there is one hundred times as much at pH 8.0 as is present at pH 6.0. Such a difference might be expected to markedly change the toxicity of the alkaloid.

The effects of changes in hydrogen ion concentration of the solution upon the proportion of free undissociated base for these three alkaloids are typical of the effects on other bases having dissociation constants of the same order of magnitude. The correspondence between this relationship and the effects of hydrogen ion concentration on toxicity is evident. In the solutions of bases having dissociation constants of the order of 1×10^{-10} there is very slight variation in the proportion of free base present with the changes in hydrogen ion concentration studied, and there is very little variation in toxicity. The hydrolysis of the alkaloid with dissociation constants around 1×10^{-7} is definitely affected by a change from pH 6.0 to pH 8.0 as is also toxicity. The greatest increase in proportion of free base occurs in the group of drugs with constants around 1×10^{-3} or 1×10^{-4}, and we find that as a whole the toxicity of this group is affected by hydrogen ion concentration more than the atropine group.

Since the difference in the toxicity of the alkaloids with changes in hydrogen ion concentration show a more or less definite relationship to the differences in concentration of free undissociated base, we may infer that it is in this form rather than as salt or ion that the base is toxic for Paramoecium. The very small differences in toxicity found for the neutral compounds and weaker bases indicate that if there is any direct action of hydrogen and hydroxyl ions on the cell it is very slight in the concentrations studied. The evidence presented therefore supports the theory that variations in hydrogen ion concentration affect the toxicity of alkaloids by changing the proportion of free undissociated base in the solution rather than by an action directly upon the cell.

Since small differences in hydrogen ion concentration can have so marked an effect on the toxicity of certain drugs it is important that this should be understood in making toxicity tests on Paramoecium. The determination of lethal concentrations for Paramoecium is a common method of comparing the toxicity of drugs, but if the determinations are not made at the same hydrogen ion concentration accurate comparisons are impossible. If the solutions tested are too acid or too alkaline we introduce another element because of the toxicity of the H- and OH-ions and such determinations will be absolutely inaccurate. Care should be taken therefore to control the pH in all such investigations.

The same principle can be applied to any determinations of the action of drugs and protozoa. The activity of chemotherapeutic agents on protozoan forms will be definitely affected by hydrogen ion concentration. Similarly in bacteriology, determinations of the antiseptic strength of drugs without proper control of the hydrogen ion concentration of the solutions may give very inaccurate results. Substances found to be bactericidal in a certain concentration at pH 7.5, may be absolutely ineffective if used in the same concentration at pH 6.5. Determinations of the bactericidal strength of antiseptics should be made at accurately determined hydrogen ion concentrations, and the magnitude of the variation with known changes in hydrogen ion concentration should also be tested.

SUMMARY

1. The limits of hydrogen ion concentration within which Paramoecium can live twenty-four hours have been found to be approximately $1 \times 10^{-9.6}$ and $1 \times 10^{-5.0}$.

2. The magnitude of the effect upon toxicity of definite changes in hydrogen ion concentration has been determined for a number of alkaloids.

3. The effect of changes in hydrogen ion concentration upon toxicity has been found to vary with the dissociation constant of the base. The free, undissociated base is apparently responsible for the toxicity.

4. The effect of hydrogen ions upon toxicity is evidently due to an action upon the drug.   If there is any direct action upon the resistance of the cell it is very slight at the concentrations studied.

It is a great pleasure to me to acknowledge my indebtedness to Dr. E. K. Marshall, Jr. for the suggestion and supervision of this piece of work, and to offer him my sincere thanks for the help and advice he has given me throughout.

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THE CHEMOTHERAPY OF ANTIMONY. COMPARISON OF THE ANTIMONYL TARTRATES WITH THE ORGANIC COMPOUNDS OF ANTIMONY

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Potassium antimonyl tartrate was first described in 1631 by Adrian de Mynsicht. Its preparation may have been suggested by a powder invented by Dudley, Earl of Warwick, and composed of scammony, antimony sulphide, and potassium tartrate ground together, of which an account was given in a treatise by Cornachinus called "Methodus in Pulverem," published in Italy in 1620; or by the practice of drinking wine which had been allowed to stand in an antimony goblet.

Early in the nineteenth century it was analyzed by a number of investigators with contradictory results (1) and was assumed to have the composition $C_4H_4O_7SbK,H_2O$ (in modern notation) until the careful work of Dumas and Piria (2) showed that it was in reality $C_4H_4O_7SbK,\frac{1}{2}H_2O$. The same observers showed that the corresponding sodium salt was $C_4H_4O_7SbNa,\frac{1}{2}H_2O$ and the ammonium salt $C_4H_8O_7NSb,\frac{1}{2}H_2O$. Another hydrate of the latter with $2\frac{1}{2}H_2O$ was afterwards discovered by Berlin (3) whilst the present writers have obtained a third hydrate containing $1\frac{1}{2}H_2O$. Dumas and Piria (4) considered the barium salt to be $C_8H_8O_{14}Sb_2Ba,2\frac{1}{2}H_2O$, although in this case their figures do not seem to have been so reliable. Careful analysis of a number of preparations now shows that three molecules of water are present.

The lithium salt was described by Buchner (5) as a transparent gelatinous mass from which small crystals separated very slowly. It was found by Plimmer and Thomson (6) to have a strong trypanocidal action, but it is not clear that a crystalline speci-

men was used. The authors have obtained it in large well formed crystals.

The aniline and quinine salts appear to have been first described by Clarke (7). The former was given the composition $C_4H_4O_6.SbOH.C_6H_5NH_2$, but Yvon (8) has since described a hydrated form, $C_4H_5O_6(SbO)C_6H_7N,H_2O$, which is efflorescent and consequently of variable antimony-content. On this account he recommends the adoption of the anhydrous salt in medicine. The quinine salt was obtained as an amorphous powder, but the present writers have succeeded in crystallising it.

Serious attempts to employ antimony in medicine have been attributed to Basil Valentine, under the influence of whose teaching it came to be looked upon as a universal panacea—so much so that towards the end of the seventeenth century there were more than a hundred preparations of the metalloid in more or less common use. During the last decade, however, antimony has assumed a new importance from the use of tartar emetic in certain tropical diseases of parasitic origin. The present position was recently outlined by one of us (9) and the conclusion drawn that results of sufficient importance had been obtained in the treatment of trypanosomiasis, bilharziasis, and leishmaniasis to necessitate search for more favourable means of presenting antimony. For some time prior to this, experiments had been in progress with the object of studying (a) the effect of variation of the basic radicle in the emetics, (b) the replacement of tartaric acid by other suitable acids, and (c) the question of the relative merits of the true organic compounds of antimony and the emetics.

So far, representative specimens of these three types have been prepared, and their toxicity determined by intravenous injection in mice. The toxicological work has been undertaken by Dr. J. Trevan, of the Wellcome Physiological Research Laboratories, and the authors are much indebted to him for his kindness in devoting so much time and care to this part of the investigation. Dr. Trevan proposes to examine the precise pharmacological action of the drugs.

Trials of all the members of this series on diseases experimentally induced in rats and other animals are also in progress. For purposes of discussion the results now published may conveniently be divided thus:

1. THE SALTS OF ANTIMONYL TARTARIC ACID

It was supposed that the antimony in tartar-emetic formed part of a basic radicle antimonyl (SbO) until Clarke and Stallo (10), in 1880, isolated ortho-antimonious acid, $Sb(OH)_3$, and submitted that the emetics were derived from this by replacing two of its hydroxyl groups by the divalent group $C_4H_4O_6$. Shortly afterwards Jungfleisch (11) suggested that the reactive groups in the tartaric acid were the alcoholic hydroxyl groups, esters which still retained an acidic function being formed.

This conception has been supported by the evidence of Guntz that the formation of antimonyl tartrates is, like ester-formation, endothermic (12); by that of Adam, who showed that addition of alkali to a tartar emetic solution caused immediate evolution of heat—evidence of the presence of a free carboxyl group—and subsequently a slow liberation of antimony oxide resembling saponification (13); and by the recent work of Blanchetière, who found that the velocity of solution of antimony trioxide in tartaric acid solutions, at first great, afterwards rapidly diminished, complete solution being attained only after an extended period (14).

The chemical results of the present investigation are generally in accord with this view, and it may be noted that the aniline and glyoxaline salts are sufficiently acid in reaction to require neutralization before injection, though when crystallization in presence of a second molecule of base was attempted the acid salt still separated.

The pharmacological results are interesting and somewhat unexpected, as it has been found possible to reduce considerably the toxicity per unit weight of antimony presented (see table on p. 358). The salts examined comprised those of:

a. The alkali metals: potassium, ammonium, sodium and lithium. These showed decreased toxicity and increased

solubility in water in the order named, thus confirming Rogers' contention (15) that the sodium salt is slightly, though definitely, less toxic than the potassium salt. The authors have been unable to confirm the statement of Plimmer and Thomson (6) that the lithium salt is more toxic than the sodium salt; in their experience it has a notably low toxicity, the amount of antimony in the minimum lethal dose being two and a half times as great as that of the potassium salt and nearly double that of the sodium salt (see table). Comparative measurements of surface tension and viscosity were also made, but these showed no parallelism to toxicity.

b. The cinchona alkaloids: quinine, quinidine, hydroquinine, cinchonine, cinchonidine and quinotoxine. Of these quinine has proved much the least toxic, the toxicity per unit weight of antimony presented being only one-fifth that of tartar emetic. In this series no obvious connection exists between toxicity and solubility or other physical properties.

c. Aniline and p-*phenetidine.* Yvon (8) has claimed that the aniline salt showed low toxicity in hypodermic injection and considerable activity against trypanosomes. In our experience the aniline salt differs but little in toxicity from the potassium salt when compared over a period of eight days.

The less soluble *p*-phenetidine salt is, however, more favourable, the toxicity per unit weight of antimony presented being roughly one quarter that of tartar emetic.

d. Ethylenediamine and butylamine. Of these, the former is considerably more toxic than tartar emetic, whilst the latter is roughly one-third as toxic per unit weight of antimony presented.

e. Glyoxaline. This salt, like that of aniline, required neutralization before injection. It is roughly only one-half as toxic as tartar emetic per unit weight of antimony presented.

Generally, then, the conclusion may be drawn that by variation of the base in the antimonyl tartrates considerably decreased toxicity per unit weight of antimony presented may be attained, the most favourable salts being those of quinine and *p*-phenetidine. The differing behaviour of aqueous solutions of quinine and quinidine tartrates on boiling with antimony trioxide is

noteworthy, the quinine being transformed almost completely into the more toxic quinotoxine, whilst the stereoisomeride, quinidine, is unaffected.

2. REPLACEMENT OF TARTARIC ACID BY OTHER ACIDS

Thomson and Cushny (16) investigated the trypanocidal action of a number of combinations of antimony with other organic acids, including lactic, citric, malic, and mucic acids, and came to the conclusion that these offered no advantage over the antimonyl tartrates. Rowntree and Abel (33) tested the efficacy of sodium antimony-thioglycollate and the triamide of the corresponding acid in experimental trypanosomiasis and found them compare favourably with the other antimonials.

The results which the authors have so far obtained with other acids, show little pharmacological interest. Chemically they support strongly the accepted view of the constitution of the antimonyl tartrates, and in cases where combination has been effected more drastic treatment has been necessary, whilst the products compare unfavourably with the antimonyl tartrates from the standpoint of ready purification.

3. THE ORGANIC COMPOUNDS OF ANTIMONY

So long ago as 1911, the *m*-amino derivatives of triphenylstibine oxide and of diphenylstibinic acid, monophenylstibinic acid, and certain of their reduction products were found to have a slight trypanocidal action, but were very irritant when administered subcutaneously (17).

The use of the intravenous route marked an important advance and the extension of the Bart reaction to the aromatic antimonials (18) has stimulated interest as it provides ready means of obtaining many hitherto difficultly accessible monoarylstibinic acids.

Thus, a number of recent communications (19, 20, 21) deal with the action of the sodium salts of *p*-aminophenylstibinic acid and its derivatives in experimental spirochaete and trypanosome infections, whilst Schmidt (22) has studied a number of compounds of this type exhaustively and has presented some

of the conclusions we had reached already. The alkali salts of the arylstibinic acids were described by Hasenbäumer (23) as easily hydrolysable substances; the ammonium salts he assumed to be incapable of existence. Further examination by the Chemische Fabrik von Heyden (24) indicated that salts of the types R.SbO(OH)(OM) or R.SbO(OM)$_2$ reacted alkaline in aqueous solution and that the neutral salts contained less than one atomic proportion of the alkali metal to a molecular proportion of the stibinic acid. In the case of phenylstibinic acid Schmidt (22) has since obtained initial neutralization when the ratio Na: Sb is approximately 1:3, but on standing the solution acquires an acid reaction, and if this be progressively removed by the addition of further alkali final neutrality is only reached after about a day at ordinary temperature, when the ratio becomes approximately 1:1. He explains this by the assumption that the free acid has a condensed molecule formed from three simple molecules of $C_6H_5SbO_3H_2$, and is gradually depolymerised in solution by alkali.

The authors have determined sodium and antimony in the isolated neutral salts of phenylstibinic acid and its m-acetylamino-, p-acetylamino-, p-bromo-, and p-ethoxy-derivatives. In the acetylamino-compounds the atomic ratio Na: Sb is approximately 1:3 but in the others it is greater, reaching 1:1.27 in the case of sodium p-ethoxyphenylstibinate. Since the methods of preparation differed in the two groups, the former being precipitated by sodium chloride from a solution saturated with carbon dioxide, and the latter formed by dissolving the free acid in alkali, it appears that a partial depolymerisation has occurred in the latter case and a mixture of salts, containing more sodium than the salt of the polymerised acid, has been formed.

In no case have the salts been obtained in a crystalline condition, and the same is true of numerous parallel attempts with the potassium and lithium salts. Sodium m-acetylaminophenylstibinate is the least toxic—only one twelfth as toxic as tartar emetic per unit weight of antimony presented—whilst the corresponding p-derivative is one-tenth as toxic. The others are much less favourable.

It is of interest to record that Caronia (25) has found sodium *p*-acetylaminophenylstibinate effective in a limited number of cases of infantile leishmaniasis of the form encountered in the Mediterranean area, whilst Manson-Bahr (26) has recorded its utility in a single case of trypanosomiasis (t. gambiense). On the other hand Wallace tested its effect in a case of trypanosomiasis (32) and considered it less effective than tartar emetic, and Manson-Bahr found it of no value in bilharziasis or benign tertian malaria (loc. cit.).

4. ANALYTICAL PROCEDURE

a. The estimation of antimony in organic compounds. Until recently the only method described in the literature was that of Cahen and Morgan (27). Schmidt (22) has employed a second method, involving a modified Kjeldahl estimation. Both of these are cumbersome and tedious, so the present authors have employed a variant of the modified Lehmann method for the estimation of arsenic devised by one of them (28) which gives excellent results.

b. The estimation of sodium. This is sufficiently indicated in the experimental portion.

5. EXPERIMENTAL

Salts of antimonyl tartaric acid

Two well-known methods have been employed in the preparation of these. In the first, molecular proportions of tartaric acid and antimony trioxide are heated in boiling aqueous solution with a molecular proportion of a monacidic base or the equivalent quantity of a di- or polyacidic base. In the second, equivalent quantities of barium antimonyl tartrate and the sulphate of the base are allowed to interact in aqueous solution. Unless otherwise stated the first method has been used.

Barium antimonyl tartrate

This salt separates from water in hexagonal plates or flattened prisms containing 3 molecules of water of crystallisation, and not

$2\frac{1}{2}$ as stated by Dumas and Piria (4). Found, loss at 110° in three specimens, 7.0, 7.1, 7.0 per cent. $C_8H_8O_{14}Sb_2Ba,3H_2O$ requires H_2O = 7.1 per cent.

Ammonium antimonyl tartrate

A third hydrate of this salt has been obtained, separating from water in large flattened prisms containing one and a half molecules of water of crystallisation. It is practically insoluble in alcohol, but readily so in water, 100 cc. of water at 18° dissolving 66 grams of the hydrate.

Found, loss at 110° = 7.8 per cent. $C_4H_8O_7NSb,1\frac{1}{2}H_2O$ requires 8.2 per cent. In dried material, Sb = 40.0. $C_4H_8O_7$ NSb requires Sb = 39.7 per cent.

Lithium antimonyl tartrate

In consequence of the ready solubility of this salt, considerable difficulty was experienced in obtaining it in a crystalline condition. This was eventually accomplished by seeding the solution, concentrated to a syrup, with a fragment of the crystalline ammonium salt, and recrystallising the ill-defined solid mass which gradually formed from boiling dilute alcohol, when large glistening octahedra were obtained. These retained $2\frac{1}{2}$ molecules of water, which were given off on drying at 60°, or over sulphuric acid, in a vacuum.

Found, loss over H_2SO_4 in a vacuum = 13.5, 13.6 per cent, $C_4H_4O_7SbLi, 2\frac{1}{2}H_2O$ requires 13.4 per cent. In air dried material, C = 14.4; H = 2.35; $C_4H_4O_7SbLi,2\frac{1}{2}H_2O$ requires C = 14.3; H = 2.7 per cent. In dried material, C = 16.7; H = 1.4; Sb = 40.9 per cent. $C_4H_4O_7SbLi$ requires C = 16.5; H = 1.4; Sb = 41.2 per cent. One hundred cubic centimeters of water at 18° dissolve 180 grams of the hydrated salt.

Ethylenediamine antimonyl tartrate

This salt separates from water as a serrated mass of flattened prisms which contain a molecule of water of crystallisation and dissolve somewhat sparingly in water or in alcohol. On heating it darkens about 280°, but remains unmelted at 300°. One hun-

dred cubic centimeters of water at 18° dissolve 4.0 grams of the hydrated salt.

Found, loss at 110° = 2.4. $C_{10}H_{18}O_{14}N_2Sb_2,H_2O$ requires 2.7 per cent. In dried material, C = 19.3; H = 3.0; N = 4.5; Sb = 38.3. $C_{10}H_{18}O_{14}N_2Sb_2$ requires C = 19.0; H = 2.9; N = 4.4; Sb = 38.1 per cent.

Butylamine antimonyl tartrate

When prepared by the "boiling" method, this salt did not crystallise from water or alcohol, in both of which it dissolved readily, but separated from boiling benzene containing a little alcohol in hexagonal prisms containing three-quarters of a molecule of benzene.

Found, loss at 60° in a vacuum = 12.8. $C_8H_{16}O_7NSb, \frac{3}{4}C_6H_6$ requires 13.5 per cent. The volatile solvent was identified as benzene. The product was, however, unsatisfactory, as it persistently retained the last traces of benzene, so the alternative method of preparation was resorted to. On concentrating the aqueous solution obtained in this way to a syrup and keeping it for some time in a cold place, a radiating mass of elongated flattened prisms separated. These were recrystallised from alcohol and formed large rectangular plates containing two molecules of water, lost on drying over sulphuric acid in a vacuum, but regained rapidly on exposure to the air, the dried material being deliquescent. The hydrated substance melted at 40° (corr.), the anhydrous at 155° (corr.). 100 cc. of water at 18° dissolved 238 grams of the hydrated salt.

Found, loss over H_2SO_4 in a vacuum = 9.0. $C_8H_{16}O_7NSb$, $2H_2O$ requires 9.1 per cent. In dried material, C = 27.1; H = 4.1; Sb = 33.3. $C_8H_{16}O_7NSb$ requires C = 26.8; H = 4.5; Sb = 33.5 per cent.

Glyoxaline antimonyl tartrate

This salt separated from concentrated aqueous solution in well-defined flattened prisms containing 2 molecules of water of crystallisation. These dissolved very sparingly in alcohol and the other usual organic solvents, and, on heating, darkened but

did not melt at 300°. 100 cc. of water at 18° dissolved 46.7 grams of the hydrated salt.

Found, loss at 110° = 8.8. $C_7H_9O_7N_2Sb.2H_2O$ requires 9.0 per cent. In dried material, C = 24.1; H = 2.5; Sb = 34.1. $C_7H_9O_7N_2Sb$ requires C = 23.8; H = 2.6; Sb = 34.0 per cent. The salt was found to be somewhat acid in reaction and therefore required neutralization before injection.

Aniline antimonyl tartrate

This salt crystallised from water or from alcohol in well-formed elongated rhombic prisms containing one molecule of water of crystallisation (compare Yvon, (8)), and melted at 162° (corr). Like the previous salt it required neutralisation before injection.

One hundred cubic centimeters of water at 18° dissolve 18.5 grams of the hydrated salt.

Found, loss at 110° = 4.4, $C_{10}H_{12}O_7NSb,H_2O$ requires 4.5 per cent. In dried material, C = 31.5; H = 3.2; Sb = 31.5. $C_{10}H_{11}O_7NSb$ requires C = 31.7; H = 3.2; Sb = 31.8 per cent.

p-Phenetidine antimonyl tartrate

This salt separated rapidly from water, in which it is only sparingly soluble, as a felted mass of needles. It dissolved more readily in hot alcohol, from which it formed masses of needles containing one molecule of water of crystallisation. The hydrated salt melts and effervesces at 148°, and the anhydrous salt at 245° (corr.). 100 cc. of water at 18° dissolve 8.5 grams of the hydrated salt.

Found, loss at 100° in a vacuum = 4.7. $C_{12}H_{16}O_8NSb,H_2O$ requires 4.1 per cent. In dried material, C = 34.4; H = 3.9; Sb = 28.1. $C_{12}H_{16}O_8NSb$ requires C = 34.1; H = 3.8; Sb = 28.4 per cent.

Quinine antimonyl tartrate

This was originally prepared by Clarke (7) who did not, however, obtain it in a crystalline condition. Employing the same method—that of double decomposition between quinine sulphate and barium antimonyl tartrate—it has now been ob-

tained in slender glistening needles containing a molecule of water of crystallisation. One hundred cubic centimeters of water at 18° dissolve 1.01 grams of the hydrated salt, although solution was slow, and agitation for several days was found to be necessary.

Found, loss at 110°, 3.3, $C_{24}H_{29}O_9N_2Sb,H_2O$ requires 2.8 per cent. In dried material, C = 47.2; H = 4.8; Sb = 19.6. $C_{24}H_{29}O_9N_2Sb$ requires C = 47.4; H = 4.8; Sb = 19.7 per cent.

When quinine tartrate was boiled in aqueous solution with antimony trioxide, an amorphous yellow product was obtained. This dissolved much more readily in water than quinine antimonyl tartrate and did not crystallise. Its colour, increased toxicity, and the well known methods of converting quinine into quinotoxine indicate that it is probably quinotoxine antimonyl tartrate. The solution resulting from the interaction on concentration first deposited a small crop of crystals which proved to be quinine tartrate. On further evaporation, an oil separated which was obtained solid by boiling with a little alcohol but resisted all attempts at crystallisation.

Found, loss at 60° in a vacuum = 4.2. $C_{24}H_{29}O_9N_2Sb$, $1\frac{1}{2}H_2O$ requires 4.2 per cent. In dried material Sb = 20.5. $C_{24}H_{29}O_9$ N_2Sb, requires Sb = 19.7 per cent.

Quinidine antimonyl tartrate

Unlike quinine, quinidine shows no tendency to transformation, and the same product is obtained from both processes. The salt separates from much water in slender glistening needles containing 4 molecules of water of crystallisation. One hundred cubic centimeters of water at 18° dissolve 0.31 gram of the hydrated salt. Compare Hesse (34).

Found, loss at 110° = 10.0. $C_{24}H_{29}O_9N_2Sb$, $4H_2O$ requires 10.5 per cent. In dried substance, C = 47.1; H = 4.9; Sb = 19.6. $C_{24}H_{29}O_9N_2Sb$ requires C = 47.3; H = 4.8; Sb 19.7 = per cent.

Cinchonine antimonyl tartrate

Two hydrates of this salt have been described by Traube, the one $(C_{23}H_{27}O_6N_2Sb)_2,3H_2O$, crystallising in monoclinic hemi-

morphs (29), the other $(C_{23}H_{27}O_8N_2Sb)_2,5H_2O$, in the hexagonal system (30).   The authors have obtained a third, $(C_{23}H_{27}O_8N_2Sb)_2$, H_2O, crystallising readily from water in well-formed rectangular plates.   100 cc. of water at 18° dissolve 2.96 grams of the hydrated salt.

Found, loss at 60° in a vacuum $= 1.3$ per cent. $(C_{23}H_{27}O_8N_2Sb)_2$, H_2O requires 1.5.   In dried material, C $=$ 47.4; H $=$ 4.7; Sb $=$ 20.9. $C_{23}H_{27}O_8N_2Sb$ requires C $=$ 47.6; H $=$ 4.7; Sb $=$ 20.7 per cent.

Cinchonidine antimonyl tartrate

Cinchonidine sulphate, found by analysis to be $(C_{19}H_{22}ON_2)_2$, $H_2SO_4,6H_2O$, was used in this experiment and converted into the antimonyl tartrate by double decomposition with barium antimonyl tartrate.   The aqueous solution so obtained was concentrated to low bulk, treated with alcohol until the resulting oil redissolved, and set aside for several days in a vacuum over sulphuric acid, when large rectangular plates gradually formed.   These proved to have the composition $(C_{23}H_{27}O_8N_2Sb)_2$, $5H_2O$, and melted at 192° (corr.).   One hundred cubic centimeters of water at 18° dissolve 1.37 grams of the hydrated salt.

Found, loss at 60° in a vacuum $=$ 6.7. $(C_{23}H_{27}O_8N_2Sb)_2,5H_2O$ requires 7.1 per cent.   In dried material, C $=$ 47.2; H $=$ 4.8; Sb $=$ 20.6, 20.9 per cent. $C_{23}H_{27}O_8N_2Sb$ requires C $=$ 47.6; H $=$ 4.7; Sb $=$ 20.7 per cent.

Hydroquinine antimonyl tartrate

Hydroquinine sulphate, which proved to have the composition $(C_{20}H_{26}O_2N_2)_2H_2SO_4,6H_2O$, was converted into the antimonyl tartrate and the product isolated as in the case of the cinchonidine salt.   The air dried material retained 5 molecules of water of crystallisation, and melted at 201° (corr).   100 cc. of water at 18° dissolve 2.24 grams.

Found, loss at 60° in a vacuum $=$ 12.8. $C_{24}H_{31}O_9SbN_2,5H_2O$ requires 12.8 per cent.   In dried material, C $=$ 47.3; H $=$ 4.8; Sb $=$ 19.4. $C_{24}H_{31}O_9N_2Sb$ requires C $=$ 47.1; H $=$ 5.1; Sb $=$ 19.6 per cent.

The organic compounds of antimony

These compounds were all prepared by interaction of diazonium salts with sodium antimonite. The mechanism of the reaction has been discussed recently by Schmidt (22); details of preparation of such substances as have been already described are therefore omitted.

Neutral sodium salt of m-acetylaminophenylstibinic acid

This was obtained as a pale pink amorphous powder which gave clear neutral solutions in water and alcohol. It was dried at 60° in a vacuum for analysis.

The figures obtained correspond to the formula

$$(C_2H_3O, NHC_6H_4 \ SbO)_3, O_2 \ (OH)(ONa),$$

so that the substance isolated under these conditions appears to be the sodium salt of a polymerised acid formed from three molecules of the acid $C_2H_3O,NHC_6H_4 \ SbO_3H_2$ the existence of which has been indicated by Schmidt (22).

Found, Na = 2.65; Sb = 40.49 per cent., atomic ratio Na : Sb = 1: 2.9. $C_{24}H_{25}O_{10}N_3Sb_3Na$ requires Na = 2.56; Sb = 40.21 per cent; Na : Sb = 1 : 3.

In this and other cases it was found necessary to correct the results for the presence of admixed sodium chloride, which persisted after repeated extraction with anhydrous methyl alcohol.

Neutral sodium salt of p-acetylaminophenylstibinic acid

This was prepared from p-aminoacetanilide (18,24), and formed a faintly coloured granular powder, readily soluble in water or in methyl alcohol, which differed but little in toxicity from salvarsan. Figures comparable with those for the antimonyl tartrates are given in the table.

It was dried at 60° in a vacuum for analysis and was found to contain nearly three atomic proportions of antimony to one of sodium.

Found, Na = 2.81; Sb = 40.82 per cent; atomic ratio Na: Sb = 1 : 2.8. $C_{24}H_{25}O_{10}N_3Sb_3Na$ requires Na = 2.56; Sb = 40.21 per cent; ratio Na : Sb = 1 : 3.

Neutral sodium salt of p-bromophenylstibinic acid

A solution of 8.6 grams of p-bromoaniline in 180 cc. of water containing 12.2 cc. of hydrochloric acid (D = 1.16) was diazotised at 0° with 3.5 grams of sodium nitrite, and added to a cooled mixture of 7.2 grams of antimony trioxide dissolved in 35 cc. of concentrated hydrochloric acid and mixed with 40 grams of sodium hydroxide in 100 cc. of water containing crushed ice. No evolution of nitrogen occurred until the temperature had risen to 11°. The mixture was then further diluted with water and stirred mechanically for several hours, the temperature being kept between 11° and 15°C. The filtrate from the reaction product was decolorised with charcoal, made nearly neutral to litmus with dilute sulphuric acid, filtered from a small quantity of dark solid, and since a preliminary experiment had shown that the acid was precipitated at this stage by carbon dioxide, dilute acid was added until the reaction was just neutral to congo red. The pale buff precipitate was washed very thoroughly and ground with sufficient dilute sodium hydroxide to produce a neutral solution, this solution treated with charcoal, filtered and evaporated to dryness. The residue was extracted with cold dry methyl alcohol, and the clear filtrate evaporated to dryness in a vacuum. The product so obtained was readily soluble in water and methyl alcohol to a clear solution, neutral in reaction.

Here the ratio of sodium to antimony was found to be higher than in the previous cases. Partial depolymerisation and consequent formation of a mixture of sodium salts had therefore occurred during the solution of the p-bromophenylstibinic acid in alkali.

Found, Na = 4.89; Sb = 34.71; atomic ratio Na : Sb = 1: 1.35. A mixture of 36.8 per cent of $(C_6H_4BrSbO)_3O_2(OH)(ONa)$ and 63.2 per cent of $C_6H_4BrSbO(OH)(ONa)$ requires Na = 5.04; Sb = 35.51 per cent, and ratio Na : Sb = 1 : 1.35.

Neutral sodium salt of p-ethoxyphenylstibinic acid

A solution of 13.7 grams of p-phenetidine in 250 cc. of water containing 24.5 cc. of hydrochloric acid (D = 1.16) was diazo-

tised at 0° with 7 grams of sodium nitrite. The resulting solution was added to a cooled mixture of 14.4 grams of antimony trioxide dissolved in 70 cc. of hydrochloric acid (D = 1.16) and 80 grams of sodium hydroxide in 450 cc. of water containing crushed ice. The evolution of nitrogen was slow. Six hundred cubic centimeters of water were then added and the mixture stirred mechanically for several hours. A considerable proportion of tar formed. This was removed and the filtrate decolorised with charcoal, almost neutralised to litmus with dilute sulphuric acid and saturated with carbon dioxide. The white solid which separated was found to be insoluble in water but soluble in dilute alkalis, dilute mineral acids reprecipitating it in a gelatinous form. It was dissolved to a neutral solution by careful addition of normal sodium hydroxide, evaporated to dryness under diminished pressure and the residue extracted with dry methyl alcohol. The extract was evaporated and the residue again dissolved in water, a slight turbidity removed by filtration, and the solution obtained finally evaporated under diminished pressure and dried. The yield was only small— 1.25 grams of a pale yellow solid. The filtrate remaining after saturation with carbon dioxide only yielded a trace of solid when saturated with sodium chloride.

Found, Na = 5.91; Sb = 39.37 per cent; atomic ratio Na : Sb = 1 : 1.27. A mixture of 29.75 per cent of $(C_2H_5O.C_6H_4.SbO)_3$ $O_2(OH)(ONa)$ and 70.25 per cent of $C_2H_5O.C_6H_4SbO(OH)$ (ONa) requires Na = 5.95; Sb = 39.43 per cent; Na : Sb = 1 : 1.27.

Neutral sodium salt of phenylstibinic acid

The corresponding acid was prepared substantially by the method described by Schmidt (22), and converted into the sodium salt by solution in sufficient sodium hydroxide to form a neutral solution and evaporation to dryness. The ratio of sodium to antimony showed that there also some depolymerisation had taken place.

Found, atomic ratio Na : Sb = 1 : 2.06. A mixture of 75.35 per cent of $(C_6H_5SbO)_3O_2(OH)(ONa)$ and 24.65 per cent of $C_6H_5SbO(OH)(ONa)$ requires Na : Sb = 1 : 2.06.

6. METHODS OF ANALYSIS

a. The estimation of antimony in organic compounds

Direct titration with standard iodine can be employed only in the case of compounds of trivalent antimony in which the remainder of the molecule does not absorb iodine, such as certain of the antimonyl tartrates. Many compounds require, however, preliminary destruction of the organic portion and to effect this the method of Cahen and Morgan (27) was first used. The results were somewhat inconsistent and the method is necessarily somewhat tedious so a variant of the modified Lehmann method employed by one of us in the case of arsenic (28) has been adopted, with success. With care quite concordant results can be obtained and these in the case of tartar emetic are in close agreement with the direct iodine titration. The details are as follows:

0.2 gram of the finely powdered substance is intimately mixed in a dry 300 cc. flask with one gram of finely powdered potassium permanganate, and 10 cc. of 50 per cent sulphuric acid are added quickly whilst shaking the flask. A second gram of permanganate is then introduced, followed by 10 cc. of concentrated sulphuric acid in small portions.

After the mixture has been allowed to stand for a few minutes, 10 cc. of water are added and the mixture then boiled for half an hour under a simple reflux condenser formed by a wide tube with a bulb to rest on the neck of the flask. Hydrogen peroxide is then added in slight excess, followed by 30 cc. of water, and the solution again boiled until a drop of a dilute solution of potassium permanganate gives a faint permanent pink colour. This is discharged by a drop of a dilute solution of oxalic acid, the solution is cooled, diluted to 150 cc., 2.5 grams of potassium iodide added, the flask stoppered and allowed to stand for an hour before titration with sodium thiosulphate N/10. A yellow colour often persists after all the iodine has disappeared, so that starch must be used as indicator. A control should be carried out simultaneously with the antimony compound omitted and the result corrected accordingly. The following figures were

obtained with a sample of crystallised tartar emetic air-dried without losing the transparency of the crystals.

By permanganate method: Sb = 36.1; 36.2; 36.1 per cent. By iodine titration: 36.1; 36.3 per cent. $C_4H_4O_7SbK,\frac{1}{2}H_2O$ requires Sb = 36.17 per cent.

b. *The estimation of sodium in the sodium salts*

A weighed quantity of the substance under examination was dissolved in water and the solution acidified slightly with dilute sulphuric acid. After standing half an hour the gelatinous precipitate was filtered off, very thoroughly washed with water containing a little sulphuric acid, the filtrate re-filtered if necessary until quite water-bright, evaporated to dryness and heated to drive off the excess of sulphuric acid. The residue contained traces of insoluble material, and to remove this it was extracted with boiling water and the extract filtered. Barium chloride was then added and the sulphate estimated in the usual way.

7. TOXICITY DETERMINATIONS

These measurements, as already stated, were made by Dr. J. Trevan of the Wellcome Physiological Research Laboratories. The substances were dissolved in normal saline, sterilised[1] and injected into the tail-vein of mice. The mice were then kept under observation for at least eight days. The figures given under the heading M.L.D. in the table (below) represent the dose in grams per kilo of body weight necessary to cause death in eight days. The principal chemical and physical data relating to the substances are also shown.

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[1] On one or two occasions the formation of small quantities of a crystalline substance corresponding in appearance and antimony content with sodium pyroantimonate, $Na_2H_2Sb_2O_7,6H_2O$, has been observed during the sterilization of sodium p-acetylaminophenylstibinate in normal saline solution. This is apparently inhibited by the addition of a small quantity of sodium hydroxide solution. The observation is of interest in view of the experiments of E. Schmitz (31) on the stability of partially neutralized arylarsinic acids, but the comparatively small quantity of material available has so far prevented further examination of the phenomenon.

NO.	NAME	FORMULA	PER-CENTAGE ANTIMONY	100 PARTS WATER DISSOLVE AT 18°	VISCOSITY OF 7.5 PER CENT SOLUTION AT 25°	SURFACE TENSION AT 20° DYNES/CM.	M.L.D. GRAMS PER KILO	WEIGHT OF ANTIMONY IN M.L.D. MILLIGRAMS	NO.
1	Potassium antimonyl tartrate	$C_4H_4O_7SbK,\frac{1}{2}H_2O$	36.17	7.2	1.137	73.31	0.016	5.7	1
2	Ammonium antimonyl tartrate	$C_4H_8O_7NSb,1\frac{1}{2}H_2O$	36.51	66.0	1.131	72.4	0.02	7.4	2
3	Sodium antimonyl tartrate	$C_4H_4O_7SbNa,1\frac{1}{2}H_2O$	38.01	91.0	1.177	72.74	0.025	9.5	3
4	Lithium antimonyl tartrate	$C_4H_4O_7SbLi.2\frac{1}{2}H_2O$	35.75	180.0	1.134	71.5	0.04	14.3	4
5	Barium antimonyl tartrate	$C_8H_8O_{14}Sb_2Ba.3H_2O$	31.63	0.49			0.01	3.2	5
6	Quinine antimonyl tartrate	$C_{24}H_{29}O_9N_2Sb,H_2O$	19.13	0.10			0.15	31.0	6
7	Quinidine antimonyl tartrate	$C_{24}H_{29}O_9N_2Sb,4H_2O$	17.61	0.31			0.02	3.5	7
8	Quinotoxin antimonyl tartrate	$C_{24}H_{29}O_9N_2Sb,1\frac{1}{2}H_2O$	18.86	25.0	1.223	60.19	0.023	4.0	8
9	Hydroquinine antimonyl tartrate	$C_{24}H_{31}O_9N_2Sb,5H_2O$	17.11	2.24			0.03	5.1	9
10	Cinchonine antimonyl tartrate	$C_{23}H_{27}O_8N_2Sb,\frac{1}{2}H_2O$	20.46	2.96			0.05	10.2	10
11	Cinchonidine antimonyl tartrate	$C_{23}H_{27}O_8N_2Sb,2\frac{1}{2}H_2O$	19.24	1.37			0.05	9.6	11
12	Ethylenediamine antimonyl tartrate	$C_{10}H_{18}O_{14}N_2Sb_2,H_2O$	37.06	4.0	(3%) 1.054	(3%) 72.51	0.005	1.85	12
13	Butylamine antimonyl tartrate	$C_8H_{16}O_7NSb,2H_2O$	30.48	238.0			0.045	15.0	13

14	Aniline antimonyl tartrate	$C_{10}H_{12}O_7NSb,H_2O$	30.33	18.5	1.163	71.09	0.02	6.0	14
15	p-Phenetidine antimonyl tartrate	$C_{12}H_{16}O_8NSb,H_2O$	27.29	8.5	1.191	60.29	0.08	21.6	15
16	Glyoxiline antimonyl tartrate	$C_7H_9O_7N_2Sb,2H_2O$	30.87	46.7	1.115	73.18	0.045	13.8	16
17	Sodium p-acetylamino-phenylstibinate	$C_{24}H_{25}O_{10}N_3Sb_3Na$	40.12				0.133*	55.8	17
18	Sodium m-acetylamino-phenylstibinate	$C_{24}H_{25}O_{10}N_3Sb_3Na$	40.12				0.174*	69.8	18
19	Sodium phenylstibinate	Partially polymerised mixture (see text)	46.56				0.072*	33.4	19
20	Sodium p-bromophenyl-stibinate	Partially polymerised mixture (see text)	34.71				0.019*	6.6	20

* Corrected for sodium chloride; compare page 353.

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EXPERIMENTAL INQUIRY INTO THE SEDATIVE PROPERTIES OF SOME AROMATIC DRUGS AND FUMES

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In nearly all pharmacopeias, ancient and modern, there are included a group of drugs which are characterized by a very powerful odor and which have been and which are still employed as nerve sedatives and analeptics. Among the best known of such drugs are valerian, asafetida, musk and lavender. The therapeutic employment of these drugs in such conditions—and it must be admitted that they are undoubtedly often effective—is a purely empirical one inasmuch as their beneficial action has never been definitely substantiated by experimental proof in the laboratory. The only one which has received some attention on the part of experimenters is valerian root, but the experimental data on hand concerning this drug are not adequate in explaining its sedative effects. Thus, Binz (1) made a few experiments on frogs by injecting them with oil of valerian and studying their reflex response to peripheral applications of weak acids. He found that injections of the ethereal oil finally paralyzed the nervous system. That is however an effect produced by injections of nearly all volatile oils and is not characteristic of valerian. The effects of intravenous injections of valerian preparations on the circulation have also been studied by various writers. Thus, Pouchet and Chevalier (2) on injecting oil of valerian in mammals obtained first a primary stimulation of the circulation followed by a paralysis of the heart. Bock (3) injected intravenously an infusion of valerian root, which produced a marked depression of the heart and Mayer (4) on injecting valeric acid noted that it produced the same effects as propionic and butyric acids,

namely, primary nervous excitement of the animal followed by coma and death. Here again the effects of the drugs were not specifically characteristic of valerian alone and certainly were not adequate in explaining the therapeutic results obtained clinically. Equally unsatisfactory is the most pretentious work on the subject, that of Kionka (5) who studied both the galenical preparations of valerian and salts of valerianic acid by injecting the same into animals and observing the effects on the circulation. He, also, concluded that the effect on the circulation is a primary excitation followed by a paralysis of the heart.

In connection with a study of the effect of various drugs on the behavior of rats in the circular maze it occurred to the authors that possibly the mode of action of the above drugs which are described by most modern writers on pharmacology as "often effective, presumably by olfactory and psychic reflexes" might be studied in a new way. It was thought possible that these substances exert their therapeutic effects not by being first absorbed into the circulation, but through a direct stimulation of the olfactory sense organs and through olfactory organs reflexly affecting other portions of the brain. Accordingly this hypothesis was put to the test in the present investigation.

METHOD

The mode of experimentation has been fully described by the authors in previous papers (6). Young adult albino rats are trained in the circular maze, which briefly consists of a series of concentric circular runways with communicating passages and cul-de-sacs, through which the animal is taught to thread its way to the center of the apparatus where food is placed. Healthy animals of good stock usually learn the maze problem within two weeks and are able to find their way quickly, without hesitation and without committing any errors, to the center of the maze. After the animals have been trained and their normal running time and behavior has been ascertained in a given experiment, they are treated with the drugs to be studied and the subsequent behavior is again observed.

In the present research a total of about 60 rats were trained and studied and the results obtained are expressed in the tables below.

The substances investigated were as follows: tincture of valerian, tincture of asafetida, tincture of musk, compound tincture of lavender, extract of violets and oil of roses. In addition to these a large number of samples of incense were studied to be described below. In studying the effects of the above drugs, a simple procedure was adopted. A rat was placed under a large glass funnel, in the neck of the funnel a wad of raw cotton was inserted and a few drops of the aromatic drug were poured on the cotton from the inside of the funnel. The funnel was so placed as to leave an appreciable space at the bottom for the free circulation of the air. The normal running time and behavior of a rat in the maze was first noted, then the animal was placed under the funnel and the observations were repeated at the end of fifteen minutes or longer. Control experiments were made by placing rats under funnels of the same size with wads of cotton in some cases not saturated with any drugs, in other cases soaked with water, and in still other cases impregnated with small amounts of ethyl alcohol. It may be stated at once that the control experiments gave negative results, in other words the confinement of the rats under the funnels had no effect of itself on the running time or the number of errors made. A few experiments were performed in a different way, rats were placed in metal boxes through which a forcible current of air was circulated by means of a pump. In order to study the effects of odors, the current of air was first passed through a tube containing a given drug. The results obtained by the two methods were the same. In some of the experiments with valerian and asafetida a little of the tincture was brushed on the noses of the animals instead of placing the rats under the funnels.

About 30 experiments were performed with this drug; the results are tabulated in table 1. It will be noted that in almost

TABLE 1

EXPERIMENT NUMBER	DRUG	BEFORE DRUG		FIRST READING		LATER READING		EFFECT	REMARKS
		Time	Number of errors	Time	Number of errors	Time	Number of errors		
1	Valerian	22	1	21	1	25	0	None	Old tincture
2	Valerian	15	0	54	4	63	3	Depression	
3	Valerian	21	0	17	0	18	1	No effect	Old tincture
4	Valerian	25	0	27	0	20	0	No effect	Old tincture
5	Valerian	16	0	23	0	21	0	Slight depression	
6	Valerian	15	0	18	1			Slight depression	
7	Valerian	35	1	68	6	41	4	Depression	
8	Valerian	24	0	50	3			Depression	
9	Valerian	17	0	21	1	26	1	Depression	
10	Valerian	17	0	29	2			Depression	
11	Valerian	15	0	27	1			Depression	
12	Valerian	18	0	20	1			Depression	
13	Valerian	11	0	31	3	25	4	Depression	
14	Valerian	16	0	Stalled				Depression	Many errors
15	Valerian	15	0	38	2	33	1	Depression	
16	Valerian	11	0	50	3			Depression	
17	Valerian	16	0	19	0	19	0	Slight depression	
18	Valerian	15	0	20	0	18	0	Slight depression	
19	Valerian	17	0	22	2			Depression	
20	Valerian	15	0	43	3	47	3	Depression	
21	Valerian	12	0	17	0	127	2	Depression	Very prolonged effect
22	Valerian	16	0	21	1			Depression	
23	Valerian	17	0	133	10			Depression	
24	Valerian	16	0	22	0			Slight depression	
25	Valerian	17	0	42	2			Depression	

26	Valerian	16	0	68	4	Depression
27	Valerian	21	0	40	5	Depression
28	Valerian	14	0	29	2	Depression
29	Valerian	15	0	38	1	Depression
30	Valerian	15	0	143	7	Depression
31	Control (air)	15	0	15	0	No effect
32	Control (air)	17	0	16	0	No effect
33	Control (air)	20	0	19	0	No effect
34	Control (air)	18	1	20	0	No effect
35	Control (air)	26	0	26	1	No effect
36	Control (air)	14	0	15	0	No effect
37	Control (air)	17	0	19	0	No effect
38	Control (air)	22	0	22	0	No effect
39	Control (alcohol)	16	0	17	0	No effect
40	Control (alcohol)	19	0	17	0	No effect

all of the cases a distinct depression was produced by the inhalation of valerian. In a few cases the depression was but slight and in only three cases out of the 30 was there no change produced in the behavior of the rats. In these three experiments a very old tincture was used which evidently was less efficacious than the fresh tincture employed in the other experiments. That the sedative effect was not due to the inhalation of small quantities of alcohol was proven by controls made with ethyl alcohol alone. Small amounts of alcohol could not be expected to produce much effect by inhalation anyway in view of the results obtained by the injection of ethanol published elsewhere (7).

EFFECT OF ASAFETIDA AND OTHER DRUGS

The results obtained with the other liquid preparations are shown in table 2. Asafetida was found to be also sedative to the rats and perhaps even more than tincture of valerian. A distinct depressant effect was noted on rats placed under a funnel which was treated with asafetida a day or two before and which was thoroughly washed out so that a faint odor of the drug clung to the glass.

A few experiments made with lavender and musk seem also to indicate a slight sedative action.

Two agreeable perfumes were studied; the alcoholic extract of violets and attar of roses, one drop of which was dissolved in 4 cc. of mineral oil. The effects of violet perfume were not uniform; in some cases a stimulation being noted, in other cases a depression. No stimulating effect was produced by the rose perfume but a distinct depression even from very minute quantities of it was noted in a few experiments.

EFFECTS OF INCENSE

The extensive employment of incense in the religious rites of the ancients and also its extensive employment in the Orient at the present time prompted an inquiry as to whether the inhalation of such fumes exerted any effect on the central nervous system. Accordingly, it was interesting to try the effects of burning differ-

ent samples of incense on the behavior of rats.   The results of these experiments are shown in table 3.   Two ingredients of Biblical incense, gum olibanum and gum galbanum were tested. Small quantities of each of these gums were heated in test tubes and the fumes allowed to be inhaled by the rats.   Control experiments made by burning cellulose (filter paper) yielded negative results.   In addition to these two samples of classical incense five specimens each of Japanese and Chinese incense preparations were studied.   These are designated in the table by the letters A to E.   It is difficult to identify the exact composition of the Chinese and Japanese samples.   The Japanese incense A is an ordinary mosquito cone; Japanese B is an incense, the fumes of which have a distinct odor of violets; Japanese incense C was labeled Hinode; Japanese incense D was one with a distinct odor of roses; and Japanese E was an ordinary joss stick, also commonly used for driving away mosquitoes.

Chinese incense A consisted simply of pieces of sandalwood; Chinese incense B was a coarsely ground powder of unknown composition; Chinese incense C a preparation known as "pyramid" yielding an agreeable odor; Chinese D consisted of Chinese joss sticks and Chinese E was a preparation consisting of incense in the form of a spiral coil.   The effects of burning the above substances were unexpected.   Neither the Biblical incense nor most of the samples of the Chinese and Japanese preparations produced any depression in the animals.   To these there were some exceptions.   The Hinode incense and the fumes of Chinese joss sticks occasionally had a depressant effect, but in most of these cases the fumes inhaled were altogether too concentrated, so that the effects of carbon dioxide and probably small quantities of carbon monoxide may have had a chance to exert a toxic effect.   On the other hand it was noticed not infrequently when a *faint* odor of Japanese and Chinese incense was inhaled that there was a distinct stimulation in the activity of the animals. This could not be attributed to an irritation or discomfort, but was actually manifested by a quicker running time through the maze.

TABLE 2

EXPERIMENT NUMBER	DRUG	BEFORE DRUG		FIRST READING		LATER READING		EFFECT	REMARKS
		Time	Number of errors	Time	Number of errors	Time	Number of errors		
1	Asafetida	17	0	77	3	20	0	Depression	
2	Asafetida	15	0	30	1			Depression	
3	Asafetida	15	0	27	2			Depression	
4	Asafetida	38	0	244	2			Depression	
5	Asafetida	33	1	29	1			No effect	
6	Asafetida	22	0	26	2			Depression	
7	Asafetida	18	0	51	6			Depression	
8	Asafetida	24	0	Entirely lost				Marked depression	
9	Asafetida	14	0	92	4			Depression	
10	Asafetida	19	0	26	0	23	3	Slight depression	
11	Asafetida	15	0	19	1			Slight depression	
12	Asafetida	19	0	56	5			Depression	
13	Asafetida	22	0	180	10			Marked depression	
14	Asafetida	16	0	21	0			Slight depression	
15	Asafetida	15	0	30	2			Depression	
16	Asafetida	20	1	180	6	53	6	Depression	
17	Asafetida	22	0	47	1			Depression	
18	Asafetida	19	0	28	0			Slight depression	Depression next day
19	Asafetida	13	0	45	2			Depression	
20	Asafetida	20	0	23	2			Depression	Drug applied to nose
21	Asafetida	14	0	67	5			Depression	
22	Asafetida	18	0	21	0			Slight depression	
23	Asafetida	18	0	25	0			Slight depression	
24	Asafetida	20	0	27	0			Depression	
25	Asafetida	16	0	37	3	16	0	Depression	

No.	Drug							Effect	
26	Asafetida	15	0	30	3		—	Depression	Very faint odor
27	Asafetida	20	0	20	0			No effect	Very faint odor
28	Asafetida	17	0	36	-1			Depression	
29	Lavandula	16	0	38	-2			Depression	
30	Lavandula	19	0	32	-1	55		Depression	
31	Lavandula	18	0	25	0			Depression	
32	Musk	20	0	42	-2			Depression	
33	Musk	16	0	34	-1			Depression	
34	Musk	15	0	43	-3	47		Depression	Effect present long time
35	Musk	21	0	22	-1		3	Slight depression	
36	Extract of violet	20	0	20	0			No effect	
37	Extract of violet	19	0	163	-5			Depression	
38	Extract of violet	17	0	14	0			Stimulation	
39	Extract of violet	27	0	20	0			Stimulation	
40	Extract of violet	20	0	27	-1			Depression	
41	Extract of violet	28	0	150	-8			Depression	
42	Extract of violet	22	0	21	0			No effect	
43	Extract of violet	23	0	25	-1			Slight depression	
44	Extract of violet	58	1	125	-6			Depression	Very weak odor
45	Extract of violet	19	0	14	0			Slight stimulation	
46	Extract of violet	25	0	129	-4			Depression	Very weak odor
47	Extract of violet	32	1	27	-1			Doubtful	Very faint odor
48	Extract of violet	18	0	Over 3 mins.	-4			Marked depression	Very faint odor
49	Extract of violet	24	0	138	-10			Marked depression	Strong odor
50	Extract of violet	19	0	Over 3 mins.	-4			Marked depression	Strong odor
51	Oil of roses	27	1	Stalled				Marked depression	Strong odor
52	Oil of roses	22	0	21	0			No effect	
53	Oil of roses	36	2	Over 3 mins.	-5			Marked depression	
54	Oil of roses	31	1	187	-5			Marked depression	Very faint odor
55	Oil of roses	21	0	81	-2			Depression	Very faint odor
56	Oil of roses	27	0	Over 3 mins.	-8			Marked depression	Very faint odor

TABLE 3

EXPERI-MENT NUM-BER	DRUG	BEFORE DRUG		FIRST READING		LATER READING		EFFECT
		Time	Num-ber of errors	Time	Num-ber of errors	Time	Num-ber of errors	
1	Olibanum	15	0	20	0			Doubtful
2	Olibanum	15	0	15	0			No effect
3	Olibanum	15	0	15	0			No effect
4	Olibanum	18	0	17	0			No effect
5	Olibanum	15	0	16	0			No effect
6	Galbanum	19	0	38	0	163	6	Depression
7	Galbanum	15	0	15	0			No effect
8	Galbanum	19	0	20	0	29	2	Depression
9	Galbanum	13	0	11	0			Doubtful
10	Galbanum	58	0	75	1	40	0	Depression
11	Galbanum	20	0	20				No effect
12	Japanese A	19	0	19	0			No effect
13	Japanese B	18	0	27	1			Depression
14	Japanese B	10	0	18	0			No effect
15	Japanese B	22	0	36	1			Depression
16	Japanese B	20	0	18	0			Stimulation (?)
17	Japanese B	19	0	19	0			No effect
18	Japanese B	20	0	18	0			Stimulation
19	Japanese B	18	0	27	1			Depression
20	Japanese B	22	0	36	1			Depression
21	Japanese B	37	0	Stalled				Depression
22	Japanese B	20	0	22	0			Slight depression
23	Japanese C	28	0	175	5			Depression
24	Japanese C	27	1	73	3	24	0	Depression
25	Japanese C	25	0	61	3			Depression
26	Japanese C	21	0	76	3			Depression
27	Japanese C	18	0	61	1			Depression
28	Japanese C	22	1	61	2			Depression
29	Japanese C	16	0	13	0			Stimulation
30	Japanese C	18	0	15	0			Stimulation
31	Japanese C	22	0	19	0			Stimulation
32	Japanese C	23	0	22	0			No effect
33	Japanese C	15	0	15	0			No effect
34	Japanese D	14	0	14	0			No effect
35	Japanese D	22	0	20	0			No effect
36	Japanese D	17	0	17	0			No effect
37	Japanese E	22	0	16	0			Stimulation
38	Japanese E	17	0	15	0			Stimulation
39	Japanese E	20	0	25	0			Slight depression
40	Control	22	0	20	1	17	0	No effect
41	Cellulose	24	0	25	0	26	0	No effect

TABLE 3—*Continued*

EXPERIMENT NUMBER	DRUG	BEFORE DRUG		FIRST READING		LATER READING		EFFECT
		Time	Number of errors	Time	Number of errors	Time	Number of errors	
42	Cellulose	15	1	15	0			No effect
43	Cellulose	15	0	12	1	14	0	Doubtful
44	Chinese A	17	0	17	0	19	0	No effect
45	Chinese A	21	0	72	3			Depression
46	Chinese A	19	0	20	0			No effect
47	Chinese A	16	0	14	0			Stimulation
48	Chinese B	20	0	37	0			Depression
49	Chinese B	19	0	15	0			Stimulation
50	Chinese B	18	0	65	3			Depression
51	Chinese C	17	0	15	0			Stimulation
52	Chinese C	14	0	14	0			No effect
53	Chinese C	17	0	25	0			Doubtful
54	Chinese C	22	0	20	0			Stimulation (?)
55	Chinese C	23	1	18	1			Stimulation
56	Chinese C	19	0	137	10			Depression
57	Chinese D	21	0	125	6			Depression
58	Chinese D	40	0	80	3			Depression
59	Chinese D	45	1	27	0			Stimulation
60	Chinese D	20	0	39	2			Depression
61	Chinese D	40	10	110	1			Depression
62	Chinese D	22	0	70	4			Depression
63	Chinese E	15	0	20	0			Doubtful
64	Chinese E	31	2	24	1			Doubtful
65	Chinese E	22	0	22	0			No effect

DISCUSSION

Of the odoriferous drugs examined the two which produced a distinct sedative effect on the behavior of rats were valerian and asafetida. A sufficient number of experiments with these drugs and proper controls have been made to render this conclusion pretty certain. Of the other preparations studied the results are doubtful. Inasmuch as in the above experiments with valerian and asafetida the amount of the drug inhaled was too small to be measured, the data obtained in the experiments cannot very plausibly be explained by the absorption of the drugs into the blood and secondary effects on the nervous and muscular systems, as in the experiments made by Binz, Kionka and other

authors. It is much more reasonable to assume that the sedative effect in the present instance is to be regarded as intimately associated with the action of the substances in finely divided form on the olfactory organs. Reflexes through the olfactory lobes probably play an important rôle in connection with the sedative effects of these drugs in various nervous conditions. It is interesting to note that of the two principal constituents of Biblical incense, olibanum and galbanum, neither one produced any physiological effect, by the methods studied above. It may be well to refer in this connection to the distinctly antiseptic action described by one of the authors elsewhere (8). The lack of depression after the use of other incense is also surprising but agrees well with the statements of some orientals, that the inhalation of the fumes of certain specimens of incense are actually stimulating to the mental processes. Finally it may be added that the inhalation of tobacco smoke is by no means as innocuous as that of incense. A few experiments were made with such smoke and the toxic effects were so marked that further experimentation was discontinued.

SUMMARY

1. The effects of a number of odoriferous substances were studied on the behavior of rats in the circular maze.

2. It was found that valerian and asafetida exert a distinctly sedative effect when studied in this way.

3. The inhalation of fumes of various samples of incense was not found to produce any depressant effect unless the fumes were so heavy as to render intoxication with poisonous gases probable.

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A STUDY OF ANTISPASMODIC DRUGS ON THE BRONCHUS

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INTRODUCTION

A very large number of drugs have been employed by clinicians for the relief of bronchial spasm, or true asthma. From an examination of the pharmacological literature on the subject it is not quite clear how the majority of such drugs exert their effect in relieving the condition. The question as to whether some of these actually produce a bronchial dilatation or whether they act on the cerebrum, on the blood, or in some other way has not yet been settled. In the present investigation the authors have attempted to determine definitely what the action of a number of so-called anti-spasmodic or anti-asthmatic drugs is on the bronchi themselves.

Among the earliest pharmacodynamic studies bronchial responses to drugs were those of Francois Frank (1) who registered the fluctuations in endopleural pressure, Lazarus (2) who studied the changes in the volume output of the lungs and Beer (3) and Einthoven (4) who recorded with a monometer the variations in intratracheal air pressure. This intricate subject was studied extensively by later writers and the various methods employed may be conveniently grouped in there classes. In the first place Dixon and Brodie (5) made oncometric studies of the lungs and similar oncometric or plethysmographic experiments were carried out by De Gamrat (6), Prevost and Saloz (7) and Golla and Symes (8). Again the endopleural method has been elaborated by Jackson (9), thirdly, Baehr and Pick (10) approached this subject by perfusion of the lungs. Lastly, Trendelenburg

(11) and Titone (12) studied the action of drugs on the bronchi by employing excised surviving bronchial rings or strips. The last method may be termed the *direct* one in contradistinction to all the three other groups, which methods may be termed *indirect* ones inasmuch as the results obtained by them are a summation of the bronchial effects plus various other modifying changes induced in the body by the particular drugs.

<div align="center">METHOD</div>

In the present investigation the authors were expecially interested in the action of drugs on the bronchi themselves, without reference to the modifications in the same produced by other organs. Accordingly, the method employed was the so-called direct one, namely the study of various drugs on the excised and surviving bronchial muscle. The procedure employed was a slight modification of that first used by Trendelenburg and may be described briefly as follows.

Trendelenburg employed the organs of the ox. In the present investigation all the experiments were made with the bronchi of the pig. This was done chiefly because such material could be most conveniently obtained in a perfectly fresh condition, some times within a half hour after killing of the animal, from an abattoir situated within a stone's throw of the laboratory. Immediately after slaughtering the animal the lungs are excised and, from these pieces of bronchi, on an average 1 cm. in diameter are cut out and washed free from mucus with Locke's solution. The bronchi are then cut into rings 0.5 cm. wide, the rings are cut open and the bronchial cartilage removed, by very careful dissection, from the combined layers of bronchial muscle and mucosa. The bronchial strips thus obtained are suspended in a small glass chamber filled with 25 or 30 cc. of warm oxygenated Locke's solution. One end of the bronchial strip is fixed at the bottom of the chamber while the free end is attached to the short arm of a lever, the long arm of which is arranged to write on a slowly moving kymograph. The small chamber containing the bronchial strip suspended in the Locke's solution is immersed in a water bath and the temperature is kept constant

at 38°C.   For the study of the reaction of bronchial muscle to physiological or pharmacological agents it is essential in the case of the bronchi, just as in the case of excised arterial rings or strips to overcome first the excessive tonic contraction of the preparation occurring after the death of the animal.   To do this a weight varying from one to five grams is suspended from the longer lever and the preparation is stretched for a period varying from fifteen to thirty minutes.   After that the "stretching weight" is taken off and a very light "lifting weight" is suspended in its place.   The lifting or balancing weight is so adjusted that the bronchial muscle writes a perfectly horizontal line on the kymograph.   This stage having been reached the preparation is ready for study.   The effects of drugs are investigated by the introduction of warm solutions directly into the chamber in which the bronchial muscle is suspended and soon after this the bronchial muscle responds by contraction or relaxation as the case may be.

PHYSIOLOGICAL CONSIDERATIONS

When a fresh bronchial preparation from a normal animal is suspended according to the method described above it will respond quickly and sharply to treatment with pharmacological agents.   As a preliminary to a study of the action of drugs on the bronchial muscle certain physiological phenomena must be considered.   In the first place even after the excessive post mortem contracture of the bronchial muscle has been overcome by the stretching weight, the bronchial preparation suspended in warm and oxygenated Locke's solution still possesses a considerable normal tonus.   This is proven by its relaxation on treatment with broncho dilator drugs as will be shown later.   Occasionally spontaneous changes in the tonus of the preparation have been noted by the authors not unlike those described by Einthoven and Dixon and Brodie, but in no case was there any rhythmic occurrence of the phenomena observed in the present investigation.

The spontaneous changes in tonicity must not be confused with the contractions or relaxations produced by changes in

temperature, for while the bronchial preparations are not as sensitive to changes in temperature as excised intestinal or uterine strips, nevertheless changes in temperature will markedly affect the tonus of the bronchial muscle. Gentle heating from 35° to 40°C. produces relaxation, but higher temperatures and the warming of preparations from lower levels of temperature may produce a contraction. Cooling ordinarily produces contraction of the bronchial muscle.

Another factor of importance is the oxygen. To obtain completely satisfactory results for pharmacological work the oxygen supply should be constant and continuous. Perhaps more important even than oxygenation and slight fluctuations in the temperature of the Locke's solution in which the bronchial preparation is suspended, are the hydrogen ion concentrations of the Locke's solution and of the drug solutions studied. This is a point which has not been carefully studied by the earlier workers on excised bronchi. It is not of much importance in connection with the study of minute quantities of alkaloid salts and other active principles, but is of paramount importance in connection with the study of the effects of various ions. Contrary to the experiences of Trendelenburg the authors have found that small doses of acids produce a contraction of the bronchial muscle and larger doses of the same increase the contractions still more, finally killing the preparation in the contracted state. Thus for instance in figure 1, small doses of $\frac{N}{10}$ hydrochloric acid added to the 25 cc. of Locke's solution in the chamber produced a distinct contraction. Further doses of the hydrochloric acid increased the contraction still more, finally killing the preparation as indicated by its failure to relax on treatment with atropin. The effect of $\frac{N}{10}$ sodium hydroxide was somewhat different while small doses of the same, for instance, 0.25 cc. in 25 cc. of Locke, produced a contraction, a further addition of the hydroxyl ions produced marked relaxation and finally death of the preparation. Chemically pure sodium carbonate in small doses produced a contraction of the bronchial muscle. Thus starting with the Locke solution of pH 7.6, the addition of 30 mgm. of sodium carbonate produced a contraction of the

muscle and a change in the hydrogen ion concentration to 8.6. Chemically pure sodium bicarbonate produced exactly the same sort of contraction but in this case 60 mgm., or twice as much of the drug was required to produce a hydrogen ion concentration of 8.6.

If the above physiological changes are controlled and a normal fresh bronchial preparation is made according to the method described above such a preparation furnishes an excellent and very sensitive test object for the study of the effects of drugs on the bronchi as will be shown below.
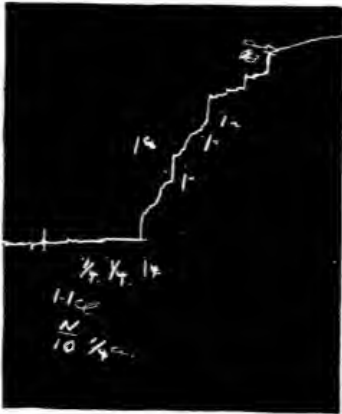
FIG. 1   FIG. 2

FIG. 1. Pig's bronchus. Repeated doses of $\frac{N}{10}$ HCl produce contraction and finally death (no response to atropin *atr.*).

FIG. 2. Pig's bronchus. $\frac{N}{10}$ NaOH first constricts then relaxes and kills (no response to muscarin).

PHARMACODYNAMIC REACTIONS

1. Drugs affecting sympathetic terminals

Of these the most important is epinephrin. This drug is known to have a remarkable therapeutic effect in many cases of bronchial spasm. Experiments with it on the isolated bronchial muscle in fact show that it produces a relaxation. In experimenting with this drug one must be very particular in controlling the hydrogen ion concentration of the medium inasmuch as
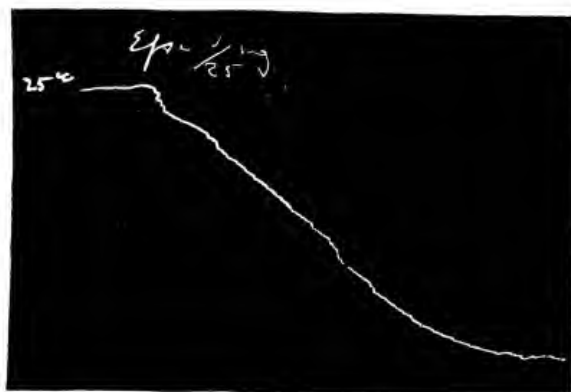
FIG. 3. Bronchus of pig.   Relaxation produced by 0.04 mgm. epinephrin after previous heightening of the tonus by muscarin.
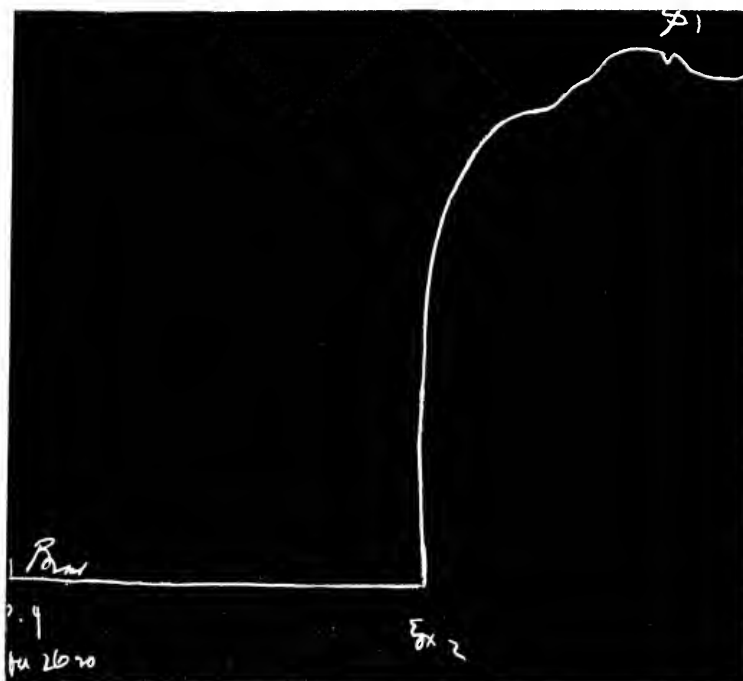
FIG. 4. Bronchus of pig.   Marked contraction produced by 2 mgm. of ergotoxin phosphate in 25 cc. of Locke's solution.

solutions of epinephrin are generally acid and unless carefully neutralized the constrictor effect of the hydrogen ions tends to antagonize the dilator effect of epinephrin.

Ergotoxin is the other drug belonging to this group which was investigated. It was found to produce a most powerful contraction of the bronchial preparations.
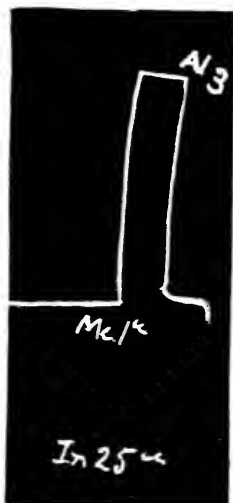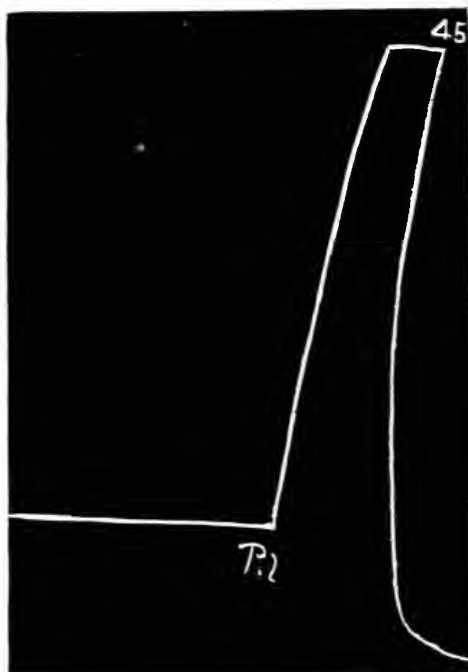
FIG. 5   FIG. 6

FIG. 5. Surviving bronchus of pig. Muscarin constricts, atropin, 3 mgm. relaxes.

FIG. 6. Pilocarpin hydrochloride, 1 mgm. produces marked contraction. This is completely relaxed by 1 mgm. of atropin sulphate.

2. Drugs affecting parasympathetic terminals

The following were studied: pilocarpin, physostigmin, muscarin, atropin, hyoscyamin and hyoscin (scopolamin). Pilocarpin and physostigmin and muscarin were all found to stimulate contraction. The broncho-constriction produced by a

saline extract of the fungus *Amanita muscaria* was so pronounced that such a solution was used as a routine practice whenever it was desired to produce a broncho-constriction of a preparation. Atropin sulphate produced prompt and marked relaxation of the bronchial muscle, so did hyoscyamin and hyoscin. On comparing the relative potency of l-hyoscyamin and d-hyoscyamin there seemed to be some indication of a more pronounced dilator effect on the part of the dextro variety as compared with the laevo. A similar relationship was noted in case of the dextro and laevo scopolamins.

3. Ganglionic poisons

Three drugs belonging to this class have been employed empirically by clinicians, namely: tobacco and extracts of lobelia

FIG. 7. Bronchus of pig.   Relaxation produced by 2 mgm. of nicotine tartrate.

inflata and of Gelsemium sempervirens. The authors experimented with solutions of nicotine tartrate, lobelin and gelsemin. It was found that all three tended to produce a slow and mild relaxation of the bronchial preparations. After small doses of the alkaloids there were indications in some of the experiments of a slight primary stimulation or contraction of the bronchial muscle, which was quickly followed by a relaxation.

4. Drugs acting on the muscle cells

Of the drugs belonging to this class the following were studied: barium chloride, papaverin, chelidonin and a number of benzyl preparations. It was found as was to be expected that barium chloride produced a powerful contraction of the bronchial muscle. Papaverin which is remarkable for its tonus-lowering

and relaxing action on all forms of smooth muscle was found to produce a rapid and marked relaxation. Hanzlik (13) has recently pointed out that chelidonin is very closely related chemically to papaverin, both of these substances being benzyl-isoquinolin compounds. It was therefore interesting to test this drug and it was found that chelidonin also produced a relaxation of smooth muscle though not as rapidly as papaverin.

Of the benzyl compounds the following were tested in vitro: benzyl alcohol, benzyl benzoate, benzyl acetate and benzyl nitrite. It was found that benzyl alcohol and benzyl acetate produced a rapid relaxation. Benzyl benzoate also produced a relaxing effect, but owing to the insolubility of the drug the action was exerted more slowly. In experimenting with benzyl alcohol solutions, as in the case of epinephrin, care must be taken to neutralize an excess of hydrogen ion concentration inasmuch as solutions of benzyl alcohol tend rapidly to oxidize and turn acid. The effect of benzyl nitrite recently studied by one of the authors (14) was a very marked relaxation, which was rather slow in setting in, but which lasted for a very long time. Inasmuch as in the case of the last drug, the nitrite ion plays a role more will be said about it in connection with the effect of nitrites.

5. Opium alkaloids

The alkaloids of the morphin group, namely, morphin, codein (methyl morphin), thebain (dimethyl morphin) and heroin were found to produce no effect on the bronchial preparations. In no case was there a definite relaxation noted, but in some cases a slight tendency to a contraction was observed. The alkaloids, papaverin, narcotin and narcein belonging to the isoquinolin group produced a marked relaxation, papaverin being much more powerful than narcotin or narcein in this respect. Administration of pantopon, a mixture of the combined opium alkaloids tended to produce relaxation, thus indicating the preponderance of the papaverin members, as far as the effect on smooth muscle is concerned.
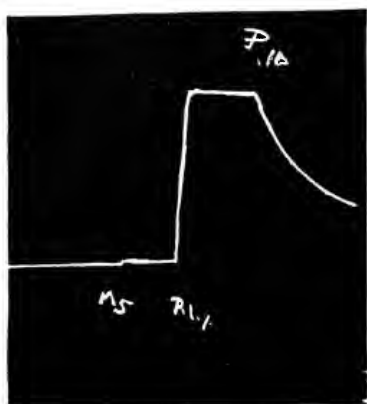
FIG. 8

FIG. 9
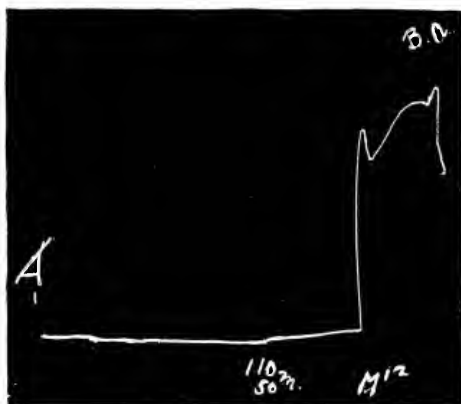
FIG. 10
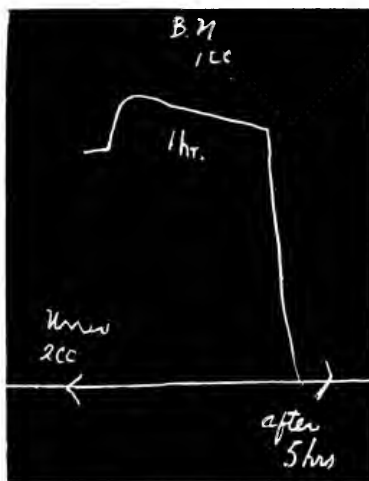
FIG. 11

FIG. 8. Bronchus of pig. *1*. Morphine sulphate, 5 mgm. (M–5) gives no effect. *2*. Pilocarpin hydrochloride, 1 mgm. (Pil–1) produces contraction. *3*. Papaverin hydrochloride, 1 mgm. (P–1) produces relaxation.

FIG. 9. Bronchus of pig. Relaxation produced by 10 mgm. of chelodonin.

FIG. 10. Bronchus of pig. *1*. Nitrate ions (NO₃) produce slight rise in tonus. *2*. Muscarin produces marked contraction. *3*. Benzyl acetate, 2 drops, produced prompt relaxation.

FIG. 11. Bronchus of pig. Tonus raised by muscarin infusion. Bronchus then treated with 1 cc. of benzyl nitrite solution 1 per cent (B.N.). Note slow relaxation. Drum was stopped at the end of one hour and started again after four hours later. Note marked effect at the end of that time.

FIG. 12

FIG. 13
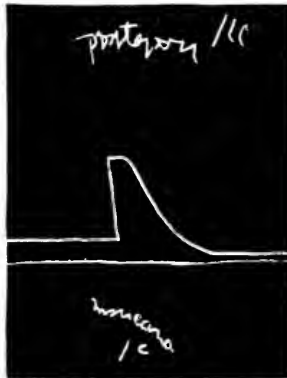
FIG. 14

FIG. 15

FIG. 16

FIG. 12. Bronchus of pig. *1.* Morphine sulphate, 5 mgm. produces slight contraction (M-5). *2.* Pilocarpin HCl, 1 mgm. increases the contraction. *3.* Benzyl alcohol (B.L.) 1 cc. of 1 per cent solution produces relaxation.

FIG. 13. Bronchus of pig. Muscaria infusion produces contraction. This is relaxed by narcotin HCl, 5 mgm. (N-5).

FIG. 14. Bronchus of pig. 1 cc. of muscaria infusion produces contraction. Pantopon, 5 mgm., produces relaxation.

FIG. 15. Bronchus of pig. Effect of 5 mgm. stovain in 25 cc. Locke's solution.

FIG. 16. Bronchus of pig. Effect of 2 mgm. of beta eucain in 25 cc. Locke's solution.

6. Local anesthetics

Some of the local anesthetics are occasionally used for the relief of asthma. It was interesting to inquire into their action on the bronchi. The following drugs of this class were studied: cocain, novocain, stovain, alypin, apothesin, benzyl alcohol, holocain and eucain. Apothesin was found to have very little effect on the bronchial muscle. All of the other drugs produced more or less relaxation. Of the two eucains the beta variety seemed to be a more powerful broncho-dilator than alpha eucain.

7. Purin derivatives

Strong coffee has long been known among medical practitioners as a valuable adjuvant, even a sovereign remedy, in many cases of asthma. It was therefore with especial interest that the effects of caffein were studied. The results obtained by previous investigators in this connection are somewhat divergent. Trendelenburg working with isolated bronchi found that strong solutions of caffein, 1:1000, produced a slight primary constriction which was followed by a more marked dilatation. Baehr and Pick on perfusing the lungs with a solution of caffein, 1:1000, also noted a broncho-dilatation. Such doses, or course, are heroic. Pal (15) noted a broncho-dilator effect. In the present investigation the authors studied the effects of various doses of caffein, varying from 1 to 20 mgm. in 25 cc. of Locke's solution and obtained the following results: Small doses of caffein were found in most cases to have no effect on the bronchial muscle and occasionally to produce a very slight constriction; never, however, more than of a few millimeters. After large doses of caffein but little relaxation of the normal bronchial preparation was noted. If, however, the bronchial muscle was first brought into a state of high tonus or contraction, as for instance on treatment with muscarin, the relaxing effect of a subsequent dose of caffein was more marked and the dilatation was more profound. On the whole, however, the impression produced was that caffein affected the bronchial muscle, only to a slight degree. Following the experiments

with caffein or trimethyl xanthin it occurred to the authors that it might be interesting to investigate the action on the bronchi of some other xanthin derivatives. Accordingly, experiments

FIG. 17
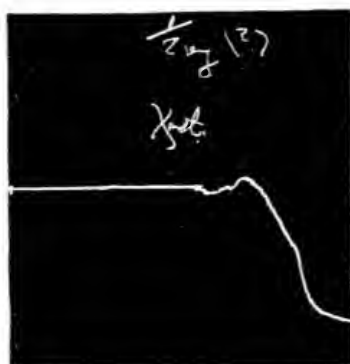
FIG. 19
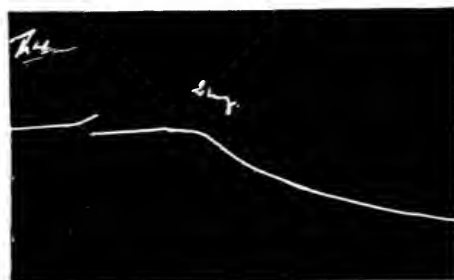
FIG. 18

FIG. 17. Bronchus of pig. Muscaria infusion (M) produces contraction. Caffein, 10 mgm., produces slight relaxation. Atropin sulphate, 2 mgm. produces more relaxation.

FIG. 18. Bronchus of pig. Theobromin or 3-7-dimethyl-xanthin, 2 mgm. in 30 cc., produces marked relaxation.

FIG. 19. Bronchus of pig. Effect of several cubic centimeters of saturated solution of xanthin, not more than 0.25 mgm. in all. Note marked relaxation.

were made with theobromin or 1-3 dimethyl-xanthin on the one hand and theocin (theophyllin) or 3-7 dimethyl-xanthin on the other hand. It was found that both of the dimethyl-xanthins produced a much greater broncho-dilatation than trimethyl-

xanthin, or caffein. It was very desirable to inquire into the effect of a mono-methyl-xanthin on the bronchus, but unfortunately the authors were unable to secure such a compound. Experiments were therefore made with xanthin itself. Xanthin is very slightly soluble in water. Nevertheless even very minute quantities of this substance (1 or 2 cc. of a solution 1:200,000) introduced into the 25 cc. of Locke's solution of the chamber produced a marked relaxation of the bronchial muscle. Hypoxanthin was also found to act in the same way. Going a step further, experiments were made with small quantities of guanin and adenin. Both of these were found to produce relaxation and were comparatively more potent even than xanthin. Passing to the nucleosid guanosin the pharmacological action became different. Guanosin produced no effect on the bronchial preparation, nor did it kill it. A few experiments were then made with adenin neucleotid and this was also found to be inactive. Finally, tests were made with solutions of thymus nucleic acid and yeast nucleic acid and these were also found to be inactive in respect to the bronchial muscle. The various compounds employed in these experiments were furnished through the kindness of Professor Walter Jones. Further work with the same is in progress.

8. Action of some ions

The effect of various ions on the isolated bronchus was studied both by Trendelenburg and Titone. The results obtained by these authors are however very different from those obtained in the present investigation. The explanation of this discrepancy is not far to seek. In the first place, those authors used entirely too large doses of the various salts studied. Thus, for instance, Titone in studying the effects of iodids introduced 5 cc. of a saturated solution of sodium iodid into his suspension chamber containing 60 cc. of Ringer's solution. He noted a dilatation of the bronchus but he himself suggests that such an effect was probably due, not to a specific action of the iodid ion, but rather to osmotic phenomena. The same author in studying the effects of sodium nitrite introduced 5 drops of 30 per cent

solution into his 60 cc. of Ringer and noted a slight relaxation. Trendelenburg also employed unnecessarily large doses of salts. Thus studying the effects of sodium nitrite he introduced 6 cc. of a 20 per cent solution into the 30 cc. of Ringer in his suspension chamber. In studying sodium nitrite 6 cc. of 1.1 per cent solution and in studying potassium chloride 6 cc. of 1.1 per cent solution were employed in the same way. In the second place the previous authors did not pay sufficient attention to the pharmaco-dynamic effects of individual ions. They experimented with ordinary solutions of the various salts in which they were interested.

In order to get a more accurate conception of the effects of various ions on the isolated bronchial muscle the present authors employed a different method. It is the method which was used by one of them (M.) in studying the effects of the iodid ion on the heart and blood vessels (16) and again in studying the effects of the nitrate and nitrite ions on the ureter (17), and furthermore employed by Macht and Hooker (18) in studying the effects of iodide bromide and nitrate ions on the respiratory center. In order to get as nearly as possible at the effect of individual ions, so-called "substituted" Locke's solutions were prepared. The normal Locke's solution employed by the present authors in their bronchial work contained 0.9 per cent of sodium chlorid together with the usual quantities of sodium bicarbonate, potassium chlorid, calcium chlorid and dextrose.

In order to study the effects of the iodid ions, part of the sodium chlorid employed in preparing the normal Locke's solution was replaced by its equimolecular weight of sodium iodid. In most of the experiments 2 grams of sodium chlorid to a liter of solution were thus substituted by its equimolecular weight or 5.13 grams of sodium iodid. In this way a so-called "substitute iodid Locke's solution" was obtained.

In order to study the effects of the bromid ions 2 grams of the sodium chlorid of normal Locke's solution in a liter were substituted by its equi-molecular weight or 3.50 grams of sodium bromid.

In order to study the effects of the nitrate ions, 2 grams of the sodium chlorid in a liter of normal Locke's solution were substituted by its equimolecular weight or 2.90 grams of sodium nitrate.

In order to study the effect of the nitrite ion, 2 grams of the sodium chlorid in a liter of normal Locke's solution was substituted by its equimolecular weight or 2.35 grams of sodium nitrite.

Lastly in order to study the effect of the potassium ion, 2 grams of the sodium chlorid in a liter of normal Locke's solution were substituted by its equimolecular weight or 2.55 grams of potasssium chlorid.

By the use of the above "substituted Locke's solutions" it was possible to ascertain more accurately the effects of individual ions to be studied and in fact it was found that the action of such substituted, balanced solutions was in some respects different from those of the ordinary solutions of the salts in water. Thus, for instance, Trendelenburg obtained a marked constriction of the bronchus by adding 6 cc. of a 1.1 per cent solution of potassium chlorid to his 30 cc. of Ringer. The present authors by employing "potassium Locke's solution" could definitely show that the effect of the potassium ion thus obtained was a distinct relaxation.

In studying the effects of the iodid ions it was found that when one-half of the normal Locke's solution was withdrawn from the suspension chamber and the same quantity of the substituted warm iodid Locke's solution was introduced in its place that a distinct relaxation was produced. On replacing the whole of the normal Locke in the suspension chamber with warm oxygenated Locke's solution a still greater and quite marked relaxation was produced in the bronchial preparation.

Experiments with a bromid Locke's solution showed that small quantities of it gave little effect. If however half or more of the normal Locke's solution in the suspension chamber was replaced by warm oxygenated bromid Locke's solution, here again the bromid ion effect was a relaxation.

Studies of the nitrate ions by the use of substitute Locke's solutions show that the nitrate ions produced no effect or in

FIG. 20. Bronchus of pig. Effect of potassium ions. One-half of the Locke's solution in the chamber was withdrawn and replaced by the same volume of a substituted Locke's solution containing equi-molecular weight of KCl in place of part of the NaCl. Note marked relaxation.
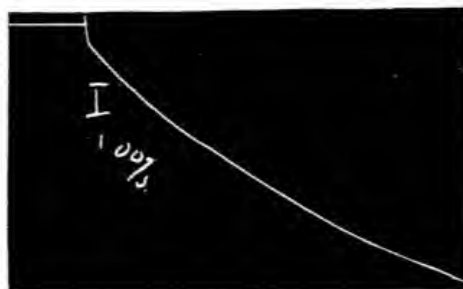
FIG. 21. Bronchus of pig. Marked relaxation produced by iodide ions through the use of Locke's solution in which 0.2 gram of NaCl in a liter was replaced by its equimolecular weight of sodium iodide.
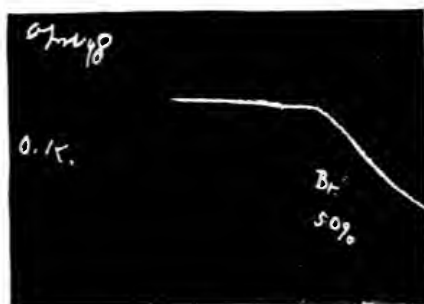
FIG. 22. Bronchus of pig. Effect of bromide ions, studied as above. Note relaxation.

some cases had a slight tendency to increase the tonus of the bronchial muscle.

The effect of the nitrite ions was interesting and remarkable. Whereas Trendelenburg on introducing 6 cc. of a 1.1 per cent solution of sodium nitrite into his 30 cc. of Ringer, obtained a contraction, experiments with substituted nitrite Locke's solutions gave very different results. It was found that when one-half of the normal Locke's solution in the suspension chamber was replaced by the same quantity of warm nitrite Locke a distinct relaxation followed. When a still greater amount of nitrite Locke's solution was introduced the relaxation was at first increased, but was gradually followed by a slow contraction of the

Fig. 23.   Bronchus of pig.   Ammonium chloride, 2 cc. of a 1 per cent solution, produces contraction which is due to the increase in the H ion concentration of the Locke.

muscle and finally by its death.   This effect was very much the same as that obtained by one of the authors on studying the action of the nitrite ions on the isolated ureter.

Inasmuch as ammonium chloride is so widely employed in respiratory conditions it was naturally very interesting to inquire into its effect on the isolated bronchus.   Trendelenburg noted a broncho-dilator effect after the use of ammonium chloride solutions.   The present authors attempted to study the effects of ammonium chloride but found it to be practically impossible by the isolated bronchus method.   It was found that as soon as ammonium chloride was dissolved in warm Locke's solution sufficient dissociation took place to raise the hydrogen ion con-

centration and inasmuch as an increased hydrogen ion concentration always leads to a contraction, no definite information as to the effects of ammonium chloride could be obtained. Thus, for instance, on dissolving 20 mgm. of ammonium chloride in 25 cc. of Locke the pH of the Locke 7.7 was changed to 7.4, which change was quite sufficient to stimulate a contraction of the bronchial muscle.

10. Action of other drugs

A large number of other drugs were studied in respect to their effects on the bronchial muscle. It was found that histamine
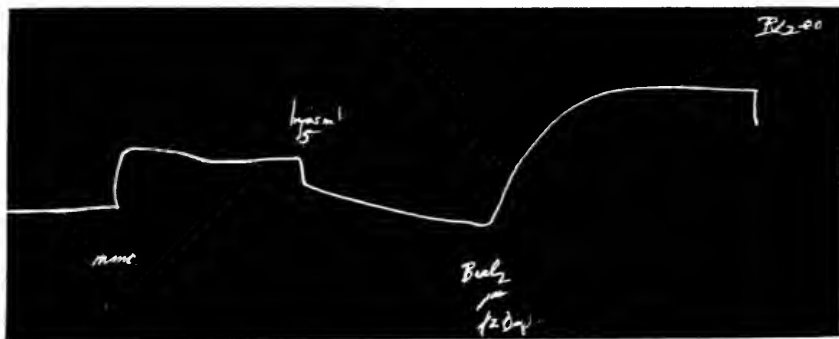
FIG. 24. Bronchus of pig. *1.* Muscaria (musc.) produces contraction. *2.* L-hyoscyamin, 5 mgm. produce relaxation. *3.* Barium chloride, 20 mgm., produces contraction. *4.* Benzyl alcohol (B.L.) 2 cc. of 1 per cent solution, produces relaxation.

produced a marked constriction of the bronchus whereas pituitary liquid produced but a slight constricting effect. Quinin salts produced relaxation. Antipyrin produced contraction. Both colchicin and colchicein produced a slight relaxation of the bronchi. Alcohol tended to relax the bronchial muscle. Ether and chloroform at first produced an irritating effect with a slight contraction which was followed by a relaxation. Urethane was also found to relax the bronchial muscle. Barium chlorid produced a marked contraction.

DISCUSSION

From the above described experiments and data it is evident
that a large number of drugs produce a relaxation of the bronchi;
some of them acting through the sympathetic apparatus, others
through the parasympathetic terminals, some through the
ganglionic elements and still others through an action on the
muscle itself. The method employed in the present investiga-
tion, by its very nature, gives us the effect of the various sub-
stances studied on the bronchial muscle and the nervous ele-
ments contained in the above, without any reference to the
modifying effects exerted through other organs, such as may
occur in the intact animal. Herein lie the advantages of this
method as well as its defects. This direct method of study
leaves no doubt as to the behavior of the bronchus itself, and
for this reason is especially suitable for comparative study of
various pharmacological agents. A comparison of the results
obtained by the direct method with a number of drugs, with the
results obtained by the indirect method, especially by the intra-
pleural method mentioned above, revealed that for the most part
data obtained are qualitatively the same, though they may vary in
degree. Wherever a qualitative difference has been noted in
the results obtained by the direct and indirect methods such
differences or apparent discrepancies could be readily explained
by the other factors playing a role in experimenting on the living
and intact animal. In the latter case when the intrapleural
method is employed, for instance, the assumption is made that
the lung is suspended in a rigid-walled cavity not unlike a plethys-
mograph. This however, is unwarranted, especially in cases
where drugs are employed which are powerful depressants of
skeletal muscle. It must be remembered that drugs may
affect the tonicity of the diaphragm and also of the muscles of
the chest wall, even when an animal is decerebrated, in such a
way as to materially change the size of the chest cavity. Again,
as has been noted by Trendelenburg, in working on the lungs in
situ it must be borne in mind that in addition to the bronchial
tree there is always present the highly ramified vascular tree

and that many drugs will affect the caliber of the pulmonary vessels, and furthermore there may be modifying influences produced by the varying pulsations of the blood vessels themselves. Again it must be taken into account that in all of the so-called indirect methods of study of the bronchi and the lungs in situ, the conditions are not much more natural than in the case of the isolated organs for in all such experiments the animals must be decerebrated or at least anesthetized, and artificial respiration must be maintained.

An examination of the various broncho-dilator drugs which were studied, shows that some of them are much more powerful in this respect than others. The present authors have found that the most rapidly acting and most powerful broncho-dilators as indicated by studies on the surviving bronchial muscle preparations are in the first place the alkaloid papaverin and secondly the benzyl compounds, benzyl acetate, benzyl alcohol, benzyl nitrite and benzyl benzoate. As far as is known all these substances seem to produce broncho-dilatation through their action on the muscle cell itself. Next in efficiency to the group of smooth muscle drugs, comes atropin. This alkaloid was also found to produce a broncho-dilatation in all cases although the action was not so rapid and not quite as intense. Third in the order of its efficiency must be placed epinephrin. This drug as is known has given most remarkable relief in many cases of bronchial asthma, and again in other cases was found, by clinicians, to be entirely ineffective. It is interesting to note that experimental data obtained with this drug by various observers are also not of uniform character. Dixon and Brodie noted no broncho-dilator effect from epinephrin in their experiments. Januschke and Pollak (19) and also Jackson observed bronchodilatation. Trendelenburg working on isolated bronchi found that adrenalin produced relaxation, while Titone using the same method, in four experiments out of twelve obtained no effect and in eight cases noted a broncho-dilatation. The present authors working on the isolated bronchus have found that when the experiment is carefully performed and when proper attention is paid to the hydrogen ion concentration of the solu-

tions used, that adrenalin produces a marked broncho-dila-
tation, though not always as powerful as the drugs already men-
tioned. In quite a number of experiments, however, in spite of
all precautions and controls, solutions of epinephrin were not
found to produce much effect on the bronchi used at all. It
must, therefore, be admitted that this drug while an efficient
broncho-dilator does not invariably produce a relaxation of
preparations of the pig's bronchi.

It is well to note in this place that a great quantitative differ-
ence in the pharmacological effect can be noted on applying
drugs to bronchi in a state of normal tonus on the one hand and
to other bronchi which have been previously brought into a
state of hypertonicity or spasm by various reagents, on the
other hand. Thus it has been repeatedly noted that while
epinephrin produces relaxation of most bronchial preparations,
such a relaxation is much sharper and more profound after
previous constriction of the bronchus, as for instance, with
muscarin. In this respect the present authors completely
agreed with the antagonism between muscarin and adrenalin
described by Januschke and Pollak.

A similar difference between normal and spastic bronchi has
been noted in their reaction to caffein. As has already been
stated caffein was found to produce but little relaxation in
ordinary bronchial preparations, but relaxation was usually
noted after previous constriction of the bronchi with some other
drug. The curious relationship between the various xanthin
derivatives and the nucleotids has been described. It is need-
less to say that the greater broncho-dilator power of the dimethyl
xanthin and of xanthin and adenin leads to the conclusion that the
therapeutic efficiency of coffee and tea in bronchial spasm is not
entirely attributable to their caffein content, but that the other
xanthin constituents always present in those beverages probably
play an important role. The advantage of the direct method of
testing small quantities of drugs on bits of bronchial muscle is
illustrated by this comparative study of the xanthin derivatives.

The effect of nicotine on the bronchi has been a subject of
contention between various observers. Einthoven found that
nicotine produces relaxation. Dixon and Brodie described

primary contraction of the bronchi which was followed by a relaxation. Titone emphatically states that nicotine produces a constriction of the bronchial muscle, while Trendelenburg noted no effect at all. The present authors experimenting with nicotine tartrate found that the effects of this alkaloid were not very striking but the conclusion drawn from all of the experiments performed is, that nicotine may produce a slight primary increase in tonus or contraction of the bronchi and this is followed by a gradual and longer-lasting relaxation.

The experiments with various ions which have been described above emphasize the importance of differentiating between the effects of individual ions and mixtures of the same, such as are present in ordinary solutions of salts. The expedient of using substituted Locke's solutions enabled the authors to determine the action of the individual bromid, iodid, nitrite, nitrate and other ions on bronchial muscle as closely as possible. It is especially interesting to note that iodid and nitrite ions produce a relaxation. The results obtained in the present experiments, however, must be greatly minimized in importance when these salts are administered to the intact animals and the bronchodilator effects of the nitrites and iodids are probably of small significance in the living body.

The authors wish to call attention also in this place, to a difference in the reaction of the bronchi in normal and pathological conditions. It has been found that while certain drugs produce definite contractions or relaxations in a normal surviving bronchus, the same drugs may produce little or no effect at all on fresh bronchial preparations from a slightly pathological lung. This point has been dealt with more fully in a separate paper (20).

Finally a few works bearing on practical medicine may not be amiss. Two tendencies of a sophistical and fallacious nature because logically unwarranted and scientifically unsubstantiated have crept occasionally into the therapeutic literature of recent years. The first is a tendency to taboo on general principles as "pharmacological superstitions," without adequate scientific proof to the contrary, the time honored and well established empirical use of a drug for the treatment of a given condition

even when the therapeutic value of that substance has been repeatedly attested by the very careful experiences of the best clinical observers. Such a practice is an extremely dangerous one and one which is liable to prove a boomerang to the critic, as illustrated by repeated instances in the recent advances of pharmacology. The other is a tendency on the part of some writers on "rational therapeutics" to underestimate the value of repeated, careful and accurate in other words scientific therapeutic or pharmacological observations on man and to regard all such data as of much less importance than data obtained from a few laboratory experiments on animals even by a tyro pharmacologist. A favorite habit of such critics is to spurn such clinical data as being the products of "psychic suggestion" or as mere "impressions" on the part of the observers or as explainable by "reflex" reactions. However ingenious, elaborate and technically masterly laboratory and animal experiments may be, if the data furnished by the same are utterly at variance with the therapeutic effects of a given drug as noted repeatedly by careful clinical observers, such evidence must perforce be accepted with a reservation and regarded as inconclusive or to say the least, incomplete; unless indeed the only alternative can be proven, namely, that the animal in question reacts to the particular drug entirely differently. If, however, the latter alternative cannot be proven, and if the same problem when approached by a different method of experimentation yields data in complete agreement with clinical experience it is but logical to conclude that the latter method of experimentation is scientifically the sounder one and the results yielded by it are the more reliable ones. It is gratifying to find that the results obtained in the present study of the effects of various drugs on the bronchi are completely in accord with the well established empirical experiences of the best clinicians, and this fact renders more valuable the data obtained by the same method with other and less well known drugs. Thus the broncho-dilator effects of epinephrin, as shown by the present method, are in complete accord with the clinical experiences with epinephrin in bronchial asthma as first described by Kaplan (21) and later by Hoover and Taylor (22), Von Jagic (23) and many other clinicians.

Again, the powerful relaxing properties of papaverin agree completely with the clinical experience of Pal (24). The remarkable relaxing action on bronchial muscle of the various benzyl compounds substantiate completely the striking results obtained by clinicians with benzyl benzoate in asthma as reported by Macht (25), Rürah (26), S. Solis-Cohen (27), Woloshin (28), W. Storm Van Leeuwen (29) and by Bullowa and Gottlieb (30) in their roentgen-ray studies on dogs. Again the empirical experiences with coffee for the relief of asthma are corroborated by the present analysis of the effects of xanthin derivatives. Finally even the empirical time-honored use of tobacco and lobelia inflata has also a modicum of scientific support as indicated by the experiments with nicotine and lobelin. The results with lobelin in the present work agree with the experimental data obtained by Dreser (31), who also noted a broncho-dilatation after that alkaloid, as indicated by an increase in respiratory volume. The action in this case is probably on ganglionic structures, inasmuch as Edmunds (32) has shown that lobelin is closely related to nicotin in its pharmacological effects.

SUMMARY

1. A large number of drugs were studied in respect to their action on the bronchi by the "direct method," that is using excised surviving bronchial preparations of the pig.

2. Relaxation of bronchial muscle may be produced by drugs either through a direct action on the muscle cells themselves or on the sympathetic, parasympathetic or ganglionic terminal structures of the bronchi.

3. The most powerful broncho-dilators determined by the above method were firstly papaverin and various benzyl compounds which act on the muscle cells, secondly atropin which exerts its action through paralysis of the parasympathetic myoneural junctions, and thirdly epinephrin which produces active stimulation of the true sympathetic dilator terminals.

4. The iodid, bromid, and nitrite ions analytically studied produced a relaxation of the bronchial muscle. This effect, however, is probably considerably minimized in the intact body.

5. An interesting chemico-pharmacodynamic relationship which is of practical interest has been traced in connection with the action of various xanthin derivatives on the bronchial muscle.

6. The action of various antispasmodic drugs on bronchial muscle varies in intensity to some extent with the previous tonicity or spasticity of the bronchus.

7. A distinct difference in response to their reaction to drugs has been found to exist between the fresh surviving bronchi from healthy lungs on the one hand and lungs showing more or less pathological change on the other hand.

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THE TOXICITY OF THE BLOOD OF ADRENALECTO-MISED FROGS

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The effects of extirpation of the suprarenal glands in frogs were first described by Abelous and Langlois (1).   More recently Loewi and Gettwert (2) claim to have shown, that the blood of adrenalectomised frogs, whether they died spontaneously or whether death was hastened by electrical stimulation of the animal by Albanese's (3) method, contains a toxic substance which has a muscarine-like action on the heart.   In their experiments they applied the blood of the decapsulated animals, soaked up in absorbent wool, to the exterior of the normal frog heart and noted after the lapse of some minutes a slowing in rate and in some cases stoppage of the heart in diastole.   These effects were removed by the exhibition of atropine, and they were inclined to attribute them to the presence of choline.   In view of the possibility suggested by Dale (4) that some substance of the type of a choline ester—such esters being more unstable and much more active than choline itself—might function as a "hormone" for the parasympathetic endings, it seemed worth while to investigate this toxic action more rigidly by perfusion experiments.

The experiments were made in the months of April, May and June.   The adrenals were destroyed by the actual cautery with aseptic precautions and under deep ether anesthesia.   Male frogs were used in most cases.   After operation the animals were kept in glass jars, which were kept moist and washed out twice daily with tap water.   The method of stimulating decapsulated frogs with faradic stimuli at two second intervals, introduced by

[1] Foularton student of the Royal Society.

Albanese and used by Loewi and Gettwert to produce symptoms
and death rapidly, was not used.   Such a procedure may easily
give rise to the production of toxic substances by stimulation of
the skin, liver or other organs, though Loewi and Gettwert claim
to have excluded such a possibility by their results in normal
frogs.

The perfusion method used was that of Hartung (5) modified
by Clark (6).   In principle it consists of a sinus cannula opening
out of a reservoir, into the open end of which the perfusion fluid
from a cannula tied into one aorta is allowed to drop.   The other
aorta and the pulmonary veins are tied off and the same fluid goes
on circulating continuously and is aerated during its fall into the
open sinus reservoir.   The perfusion system which I used had a
total capacity (including the heart chambers) of 2 cc.

The blood serum from the decapsulated frogs was obtained by
cutting out the heart and collecting the blood in a Dreyer's tube,
which was spun in a centrifuge.   Usually 0.2 cc. of serum was
added to the 2 cc. of perfusion fluid—giving a concentration of
1 in 10.

THE GENERAL RESULTS OF DECAPSULATION IN FROGS

According to Abelous and Langlois extirpation of the sup-
rarenals of frogs which are in full physiological activity causes
death in forty-eight hours, whereas if the operation is performed
on hibernating frogs they survive for from thirteen to fourteen
days, unless kept at a temperature of from 20° to 22° after the
operation.   In my experiments the temperature varied from 15°
to 19°C. during the day and the average period of survival as
shown in the attached table was six days.   In series I frogs 2, 3
and 8 died within twenty-four hours of the operation and no
obvious cause of death was found at autopsy.

Frog 6 of series 1 survived twelve days and frogs 4 and 5 of
series III ten days— in these cases extirpation was found to be
incomplete.   None of these frogs are included in the table.   Six
of the remaining fifteen frogs were found dead in the morning and
had not exhibited any symptoms other than slowing of respiration

Summary of perfusion experiments with serum of decapsulated frogs

	TIME OF SURVIVAL	SYMPTOMS AND REMARKS	EFFECT OF SERUM ON THE NORMAL FROG HEART
	days		
I-1	6	Found dead	No change in amplitude or rate of beat
4	5	Flaccid paralysis of hind limbs	No change in amplitude or rate of beat
5	7	Paresis of hind limbs—terminal convulsions	No change in amplitude. Rate changed from 32–28 per minute
7	7	Flaccid paralysis of hind limbs—no myosis	No change in amplitude or rate of beat
9	6	Found dead	No change in amplitude or rate of beat
II-1	7	Paralysis of hind limbs; fore limbs in rigor (clasped)	Transitory change in rate from 39 to 36 per minute
2	7	Flaccid paralysis of hind limbs, rigid clasped forelimbs—myosis	No change in amplitude or rate of beat
3	7	Flaccid paralysis of hind limbs, rigid clasped forelimbs—myosis.	No change in rate of beat. Temporary decrease in amplitude
4	8	Found dead, rigor mortis had set in	Not tested
III-1	4	Flaccid paralysis of hind limbs—myosis	2:1 heart block after 10 minutes; normal rhythm restored by atropine
2	5	Found dead	No change in amplitude or rate of beat
3	7	Found dead	After 11 minutes, lowered conductivity. Groups of normal beats interspersed with groups of beats showing 2:1 heart block. Atropine had no effect. Washing out with Ringer restored normal rhythm
6	5	Found dead	No change in amplitude or rate of heart beat
IV-1	3	Flaccid paralysis of hind limbs—myosis	No change in amplitude or rate of heart beat
2	6	Flaccid paralysis of hind limbs—myosis	No change in amplitude or rate of heart beat

and slight lassitude up to the previous evening. The remainder either died or were killed when moribund, and generally speaking, presented the characteristic picture described by Abelous and Langlois. Frog III-1 whose condensed protocol is here given is a typical example:

May 30, 1921. Active male frog. Respirations 146. Heart 52 per minute. 3.00–3.15 p.m. Extirpation of both suprarenals under ether anesthesia.

May 31, 1921. Respirations 144. Heart 45. No symptoms other than a slight myosis. Temperature 17° C.

June 1, 1921. Respirations 105. Unable to turn over when placed on its back. Some incoördination of muscular movements. Temperature 15.5 C.

June 2, 1921. 10 a.m. Respirations 100. Heart too feeble to count. No pulsation visible in the web. 12.30 p.m.—Paralysis of hind limbs. 2.30 p.m.—Respiration slow and irregular—myosis extreme. 4.00 p.m.—Respiration varies from 48 to 10 per minute. Conjunctival reflex very slow; lid remains up for some seconds after it is elicited. 5.50 p.m.—Frog moribund. Brain pithed. Dorsal lymph hearts still beating. 6.00 p.m.—Thorax opened. Heart beating 16 per minute. Auricles distended and ventricular contraction feeble. Left sciatic nerve exposed.

Electrical reactions. No contractions in gastrocnemius by stimulation of the sciatic with the coil at 0 cm. Muscle gives good response when stimulated directly (coil at 15 cm.)

In some cases the symptoms were atypical. Myosis was not an invariable symptom. Frogs I-4 and 7 and II-1 did not exhibit it.

Frogs II-1, 2 and 3 had rigidity in the fore limbs some hours before death, when the flaccid paralysis had appeared in the hind limbs. In frog II-1 at death this rigidity was a rigor, and the muscles did not respond to stimulation though the adjacent muscles of the floor of the mouth contracted with the coil at 40 cm.

The electrical reactions of the paralysed limbs were somewhat variable and in several cases there was no evidence of any curare-like effect. This was the case in II-2 and II-3, where immediately

after death the gastrocnemius contracted on stimulation of the sciatic nerve with the coil at 40 cm.   In all cases with well-marked paralysis the stimulation of the central end of the cut sciatic, even with the coil at 10 cm., failed to produce any reflex contraction of the opposite limb.   This sort of reaction suggests an action on the spinal centres when the muscle end-plates are still functioning and may possibly be related to failure of the circulation (vide infra).

The dorsal lymph hearts invariably went on contracting till death and there was no curare-like paralysis of these or of the eyelids.

The most striking feature of the symptom-complex next to the lower limb paralysis, is the progressive slowing of the respirations.   Usually there was slowing of the heart rate but in some instances, when the animal was pithed some hours after the onset of the paralysis, the heart was found to be beating though feebly, at little less than its normal rate.   In still other cases the auricles were found to be beating feebly, though the ventricles had ceased to do so.   When the paralysis was well marked in the lower limbs the circulation in the web was no longer evident, the capillaries were empty and there was stasis in the larger veins.

THE INFLUENCE OF THE SERUM OF ADRENALECTOMISED FROGS ON THE NORMAL ISOLATED HEART

The individual result of my experiments are given in the table· Generally speaking the serum of frogs, which were either moribund or dead as the result of adrenal extirpation, had no effect in a concentration of 1 in 10 upon the normal isolated frog's heart.   The figure shows a typical example.   In one or two instances slight temporary slowing was observed, but this effect was also obtained by a corresponding concentration of normal frog serum.   In two cases however a heart block came on ten minutes after the addition of serum to the circulating fluid.   Both these hearts were from large frogs and it is possible that the restriction of outflow through a single aortic cannula which was adapted for smaller hearts contributed to this result.   In one

case the effect was removed temporarily by the addition of
0.05 cc. of 1 in 10,000 atropine to the circulating fluid and per-
manently by the further addition of 0.1 cc.   In the other case
atropine had no effect though after washing out the system with
frog Ringer the heart reverted to its normal rhythm.   I am not
convinced that the effect of atropine in the former case is a spe-
cific one since it is not an uncommon experience to find a badly
beating isolated heart improved by treatment with atropine.
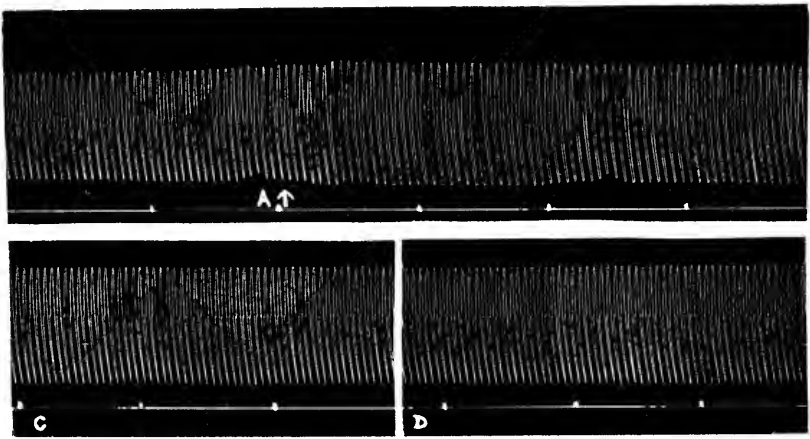
FIG. 1. PERFUSED NORMAL FROG HEART

At A 0.2 cc. serum from frog IV-1 (killed when moribund).   C, tracing ten
minutes later; D, tracing thirty minutes later.   Time in half minutes.

DISCUSSION AND CONCLUSION

Loewi and Gettwert, in the only experiment which they quote,
in which the blood of a frog dying spontaneously after extirpation
of the adrenals (without resort being had to stimulation) was
tested on the normal frog heart, obtained a slowing from 50 to
44 per minute within five minutes and a further slowing to 40
within twenty minutes.   This slowing was at once abolished
by atropine.   The perfusion method used in my experiments is a
much more accurate method than the application of serum to the
surface of the heart.   It is possible that this difference in methods
may explain the difference in results.

My experiments afford no clear evidence of the presence of any muscarine-like effect. The possible instability of the toxic substance sought for, may render suspect the perfusion experiments with the serum of frogs found dead after decapsulation, but the results were not different when the serum of animals, killed when evidently moribund, was at once tested on the isolated normal heart.

Since simple decapsulation gave no unequivocally positive results, I did not proceed to investigate the effect of stimulation, which would introduce a large number of unknown factors.

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UTERINE EFFECTS OF INTRAVENOUS INJECTIONS OF FLUIDS[1]

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The following study of the intravenous injection of fluids was undertaken with the hope of defining more clearly our knowledge of those factors which affect the uterus in the living animal. Doubtless the results are applicable to other forms of smooth muscle existing in the body under normal conditions.

Except for experiments in vitro the uterus has received but little pharmacological attention. Employing a method devised in this laboratory (1) for investigations in vivo the following points have been studied by us:

1. Salt action, including the effects of (a) hypotonic solutions: distilled water and 0.3 per cent sodium chlorid; (b) isotonic solutions: 0.9 per cent sodium chlorid and Locke's solution, NaCl 0.9 per cent, $CaCl_2$ 0.024 per cent, KCl 0.042 per cent $NaHCO_3$ 0.02 per cent; (c) hypertonic solutions: 3 per cent and 10 per cent sodium chlorid (with supplementary experiments on intestinal injections of 20 per cent sodium sulfate and 20 per cent sodium chlorid) and acacia.

2. Effects of addition or removal of blood.

3. Effects of acid and of alkali.

Changes in uterine tone and in the amplitude and rate of the rhythmic contractions were recorded by kymograph in all of the experiments. Simultaneous records were made of the blood pressure.

All experiments were performed upon healthy medium sized dogs, some of which were pregnant (indicated by "P" in the tables). The anesthetics used were morphine, 5 mgm. per kilo, followed by chloretone, 0.2 gm per kilo. All of the injections were intravenous; the amount of fluid most frequently used was 100 to 200 cc.

1. SALT ACTION

This has been investigated upon the isolated uterus by Dale (2) who found that when the osmotic tension of the solution in which the organ was suspended was lowered there was a tendency to an increased tone of the uterus, a corresponding relaxation being noted when the salt content was increased. Voegtlin (3) has also noted that distilled water increases the tone of the isolated uterus.

a. Hypotonic solutions

Distilled water. Nine injections of distilled water were made in eight different dogs. In all of these cases the tone of the uterus increased definitely, presumably due to the taking up of water by the cells. Significant changes in the amplitude and

TABLE 1

Effects of hypotonic intravenous injections

DOG NUMBER	INJECTION		UTERINE CONTRACTIONS			BLOOD PRESSURE
	Substance	Amount	Tone	Amplitude	Rate	
		cc.				
5	Distilled water	200	+ (?)	0	0	(−)?
7	Distilled water	200	++	−	+	+
8 P	Distilled water	100	++++	−	+	+
9	Distilled water	100	+++	0	0	(−)?
11	Distilled water	500	++++	0	0	
12	Distilled water	100	+	0	0	0
17 P	Distilled water	100	+++	0	0	0
17 P	Distilled water	200	+	+	+	0
18	Distilled water		+ (−)	+	+	0
5	0.3 per cent NaCl	200	+ (?)	0	0	+
6 P	0.3 per cent NaCl	200	++++	−	+ ?	−
6 P	0.3 per cent NaCl	200	++++	−	−	−
6 P	0.3 per cent NaCl	200	++	−	−	+ (?)
7	0.3 per cent NaCl	200	++	−	+	+

rate of the contractions were not always noted but the amplitude was sometimes diminished and the rate increased simultaneously with the increase in tone.   Since an increase in blood pressure was observed in but two of these experiments, two others showing a decrease, the uterine effect cannot be ascribed to changes in the circulation.

Hypotonic saline.   Sodium chlorid injections in 0.3 per cent concentration in five experiments gave results similar to those
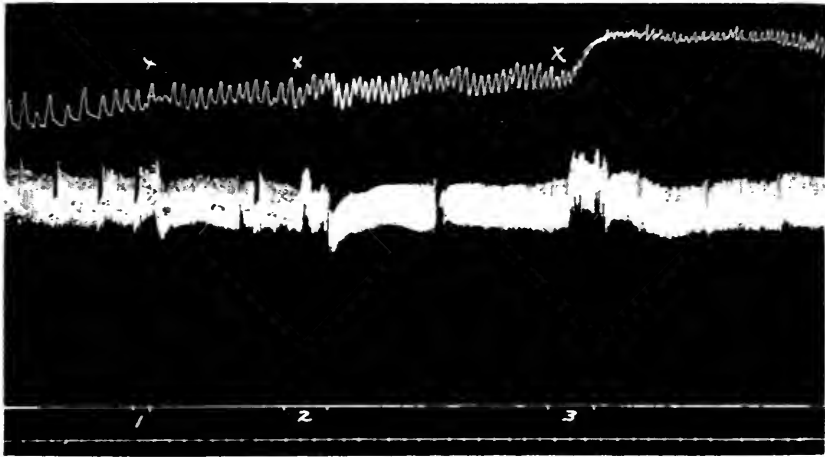
FIG. 1. Dog 7

Effects of intravenous injections upon the intact uterus.   In this and in the following figures the first line is a tracing of the action of the uterus, the second. of the carotid blood pressure. the third serves as a base line for the blood pressure and for marking the points of injection. The last line represents the time in one minute intervals.   First injection, 100 cc. 0.9 per cent NaCl; second, 200 cc. 0.9 per cent NaCl; third, 200 cc. 0.3 per cent NaCl.

seen with distilled water.   In each case 200 cc. were injected. A typical result of these injections upon the uterus is illustrated in figure 1 (third x).   The increase in the blood pressure shown here was not constant, as will be seen from table 1, which presents a summary of the effects of the hypotonic injections.   The extent of the effects is indicated here and in the later tables by the number of plus or minus signs, which represents increase or decrease respectively.   Zero indicates no effect.

b. *Isotonic solutions*

Sodium chlorid (0.9 per cent) injections were made in nine experiments on six different dogs. In the majority of these no significant effects were observed, although occasionally slight increases both in uterine tone and in blood pressure were noted. In one case 700 cc. was injected within about seven minutes in seven separate 100 cc. portions into the femoral vein, the condition

TABLE 2

Effects of isotonic intravenous injections

DOG NUMBER	INJECTION		UTERINE CONTRACTIONS			BLOOD PRESSURE
	Substance	Amount	Tone	Amplitude	Rate	
		cc.				
1	0.9 per cent NaCl	100	—	0	0	0
1	0.9 per cent NaCl	700	0	0	0	0
1	0.9 per cent NaCl	200	(−)	0	0	0
4	0.9 per cent NaCl	300	0	0	0	0
6 P	0.9 per cent NaCl	200	+	0	0	+
7	0.9 per cent NaCl	100	0	—	+	0
	0.9 per cent NaCl	200	0	—	+	0
8 P	0.9 per cent NaCl	100	(+−)	0	0	+
8 P	0.9 per cent NaCl	100	++	0	0	+
9	0.9 per cent NaCl	100	+	0	0	+(?)
1	Locke's solution	500	(−)	0	0	0
2	Locke's solution	400	(−−)	0	0	0
2	Locke's solution	100	0	0	0	0
	Locke's solution	100	0	0	0	0
17 P	Locke's solution	100	0	0	0	0
17 P	Locke's solution	200	0	0	0	0

of the uterus and of the circulation remaining unaffected. In figure 1 (first two x's) the slight changes seen in the uterine activity contrast well with the effects of the hypotonic salt solution.

Locke's solution. Six injections of Locke's solution in three different animals were practically without effect except in two cases, where large quantities (400 and 500 cc. respectively) induced some relaxation of the uterus.

The effects of isotonic solutions are summarized in table 2. The stimulation of blood pressure and of uterine tone seen

occasionally after injections of physiological saline but never after Locke's solution is presumably in some way associated with the relative excess of sodium ions.

c. Hypertonic solutions

Sodium chlorid in hypertonic concentration yielded results essentially constant whether 3 per cent or 10 per cent was used. Eight injections of hypertonic sodium chlorid were made intravenously in five different dogs. The uterus became very much relaxed in tone in every case. The characteristic increase in
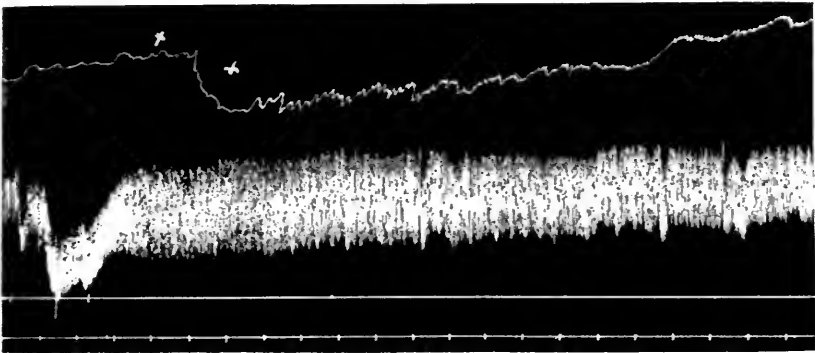
Fig. 2. Dog 4

Seventy-five cc. 10 per cent sodium chlorid injection between crosses on uterine tracing, which correspond to marks on third line.

amplitude was due apparently to attempts of the relaxed organ to attain its former level of tone. The relaxation of the uterus was usually associated with a diminution in the blood pressure, which was followed by a return to the former level considerably before the recovery of uterine tone. In one case, however, the typical effect upon the uterine tone lasted about twenty minutes without any accompanying reduction in blood pressure. Figure 2 (10 per cent NaCl) is typical of the effects of hypertonic saline injections.

Intestinal injections. Having established the fact that the injection of hypertonic saline intravenously results in a loss of

uterine tone it was deemed of interest to determine whether a similar result could be obtained under conditions in which, instead of the blood volume becoming greater at the expense of the uterus, both the blood and the uterus become dehydrated. To this end intestinal injections were made. Through an extra mid-line incision in the abdomen was inserted into the jejunum a cannula through which injections of hypertonic salt solutions were made. In figure 3 is illustrated the effect of such an injection of 200 cc. of 20 per cent sodium sulfate into the gut. A prolonged relaxation of the uterus accompanied by a slight temporary increase in the blood pressure is noted. A similar effect in which the
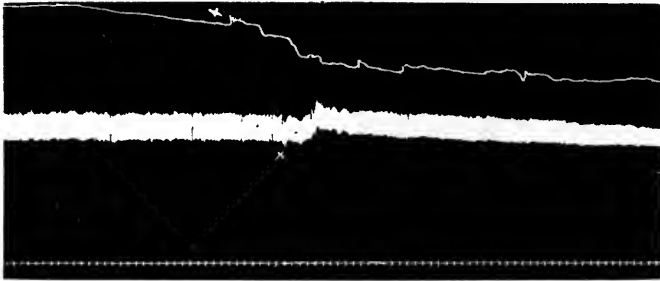
Fig. 3. Dog 18

Effects of 200 cc. 20 per cent sodium sulfate injected into jejunum. (Base line omitted.)

relaxation was even more pronounced was noted in another dog. When, however, instead of the sulfate, sodium chlorid (20 per cent) was injected into the gut (fig. 4) the first effect was usually one of contraction.

While this was possibly due to an irritation reflex the fact that the sulfate produced no such contraction may indicate that it should be attributed to the absorption of some of the more diffusible chlorid. These intestinal injections indicate therefore that where only the dehydration factor is present (sodium sulfate probably being very scantily absorbed into the circulation) the uterus simply relaxes, whereas the presence of excessive sodium ions in the blood before much dehydration takes place probably tends to promote contraction of the organ.

Acacia. As further test of the effects of increasing the osmotic tension of the blood we made a number of intravenous injections of acacia. Three strengths were used, 14, 7, and 3 per cent respectively, the gum being made up each time in physiological saline. Hanzlik and Karsner (4) have shown that the intravenous administration of acacia sometimes injures the circulatory
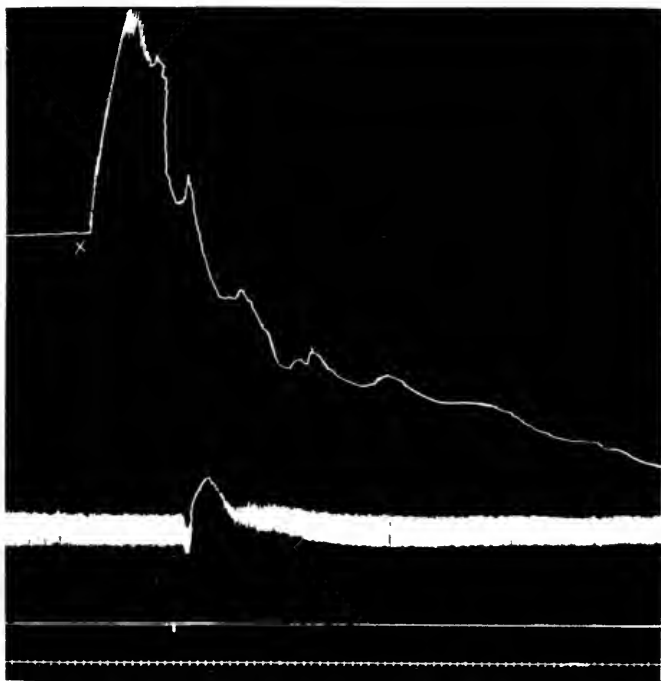
FIG. 4. DOG 18

Effects of 100 cc. 20 per cent chlorid injected into jejunum

and respiratory systems, as indicated by presence of "anaphylactoid" symptoms which they ascribe chiefly to thrombus formation. Our 100 cc. injections of acacia in the strengths mentioned did not suffice to produce circulatory embarrassment; on the contrary some temporary increase in blood pressure was usually seen.

As regards the action on the uterus itself, Hanzlik had investigated this organ in vitro. Under the conditions of his experiments acacia and other colloid substances were found to inhibit the activity of the uterus. This latter effect was seen in our experiments only in the case of a 14 per cent injection, which in

TABLE 3

Effects of hypertonic intravenous injections

DOG NUM-BER	INJECTION		UTERINE CONTRACTIONS			BLOOD PRESSURE
	Substance	Amount	Tone	Ampli-tude	Date	
		cc.				
4	10 per cent NaCl	75	— — — —	0	0	(−)++
4	10 per cent NaCl	75	— —	+*	(−)	−+
4	10 per cent NaCl	100	— — —	0	0	−+
6 P	10 per cent NaCl	100	— — —	0	0	−+
9	10 per cent NaCl	100	— — —	+*	(+)?	−+
6 P	3 per cent Nacl	200	— — —	+	0	0
7	3 per cent NaCl	100	— —	+*	0	−+
8 P	3 per cent NaCl	100	— — —	+*	0	−+
5	14 per cent acacia (in saline)	100	(−)	abol.†	abol.	++
9	14 per cent acacia (in saline)	100	(− −)	0	0	+
2	7 per cent acacia	100	0	0	0	+−
	7 per cent acacia	100	++			
	7 per cent acacia	100	+			
2	7 per cent acacia	100	+	+	−	+
5	7 per cent acacia	100	+	0	0	+
5	7 per cent acacia	100	0	abol.	abol.	+−
10	7 per cent acacia	100	+	0	0	+−
5	7 per cent acacia	100	+	0	0	+−
5	3 per cent acacia	100	0	+	?	+?
9	3 per cent acacia	100	+	0	0	+

* This characteristic amplitude increase is due apparently to attempts of the relaxed organ to attain its former level in tone.
† abol. = abolished.

addition to a small inhibition of the tone completely abolished the rhythmic activity for a time. Another injection of 14 per cent of acacia resulted in relaxing for a few minutes a uterus which had been somewhat contracted by the injection of distilled water, no definite change in the rate or amplitude of the contractions being noted. The relaxation produced by 14 per cent acacia is ascribed to increased osmotic tension of the blood.

The lower concentrations of acacia injected in 100 cc. amounts did not produce constantly any significant results except the temporary increase in blood pressure already referred to. Six out of eight injections, however, increased the tone of the uterus. This is perhaps due to the improved circulation associated with the rise of blood pressure. Of the 3 per cent injections, both of which raised the blood pressure, one injection increased the tone of the uterus and the other did not.

The results of hypertonic injections are summarized in table 3. In interpreting the effect of acacia the factor of increased viscosity as well as of hypertonicity should be kept in mind.

2. ADDITION OR REMOVAL OF WHOLE BLOOD

Having demonstrated the constant production by hypotonic injections of an increase in uterine tone and the frequency with which injections of 7 per cent acacia in physiological saline produce the same effect, it was thought advisable to test further the effect upon the uterus of increasing the bulk of the blood without altering the composition. To this end two successful transfusion experiments were performed, in each case the donor being a healthy dog anesthetized in the same way as the uterus dog. Injections of small quantities of blood in this fashion produced different results in the two animals. In the case of dog 3 (fig. 5), a pregnant animal, the transfusion of 170 cc. of whole blood over a period of seven minutes resulted in a definite increase in the blood pressure and the pulse pressure. But until circulatory collapse set in from some unknown cause at the conclusion of this injection, the only change seen in the uterus was an increase in the rate of the rhythmic contractions. In dog 4, a non-pregnant animal, the second 100 cc. transfusion yielded again a marked increase in the rate of the contractions but in this case with a simultaneous slight loss in tone. The blood pressure remained unaltered. A previous transfusion of 100 cc. in this same animal had yielded no significant result (table 4).

Increase of the blood volume by the injection of whole blood therefore produces no constant effect upon the tone of the uterus but may increase the contraction rate.
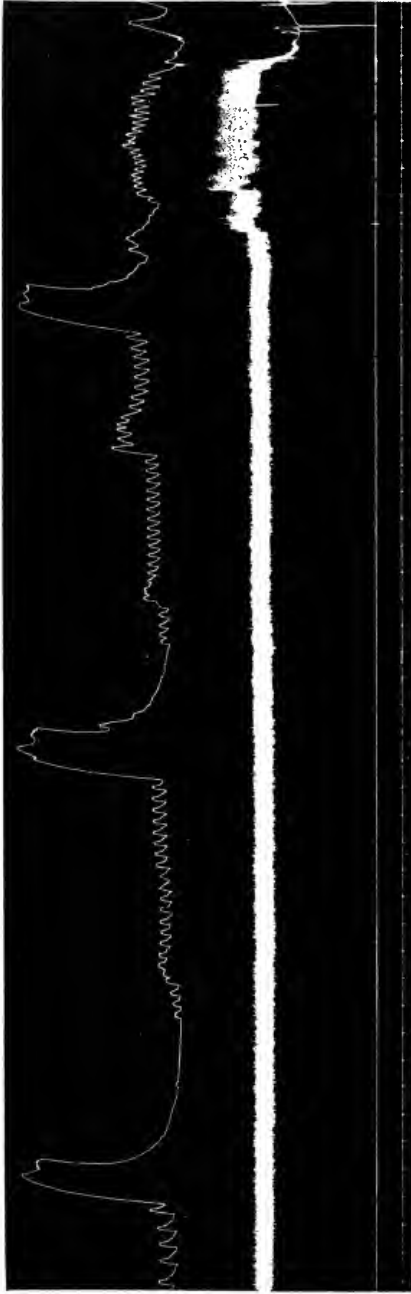
Fig. 5. Dog 3

Transfusion of 170 cc. whole blood, begun at mark on third line

Removal of blood by hemorrhage is just as ineffective in changing the tone of the uterus as the addition of whole blood. Out of nine hemorrhages varying from 50 to 500 cc. in four different dogs only one (125 cc.) altered the tone, this change being a slight relaxation. The rhythmic activity of the uterus was, on the other hand, considerably altered by hemorrhage and in each of the four dogs could ultimately be abolished. This is well illustrated in figure 6. The degree of uterine inhibition seen

TABLE 4

Effects of addition or removal of whole blood

| DOG NUMBER | PROCEDURE | BLOOD | UTERINE CONTRACTIONS | | | BLOOD PRESSURE |
			Tone	Amplitude	Rate	
		cc.				
3 P	Transfusion	+ 170	0	0	+	+
4	Transfusion	+ 100	+	0	0	− +
4	Transfusion	+ 100	− (?)	+	+	0
1*	Hemorrhage	− 150	0	0	0	0
	Hemorrhage	− 400	0	− sl.†	− sl.	− sl.
	Hemorrhage	− 500	0	abol.‡	abol.	− −
2	Hemorrhage	− 125	sl. −	0	0	(?)
2	Hemorrhage	− 100 ?	0	abol.	abol.	−
5	Hemorrhage	− 175	0	abol.	abol.	0
	Hemorrhage	− 230	0	no recov.§	no recov.	0
8 P	Hemorrhage	− 90	0	abol.	abol.	− −
	Hemorrhage	− 50	0	no recov.	no recov.	0

* After 800 cc. fluid.

† sl. = slight.

‡ abol. = abolished.

§ no recov. = no recovery of the abolished contractions.

after each hemorrhage is roughly proportional to the extent of the fall in blood pressure. It may probably be assumed that the activity of the uterus is inhibited by the poor nutritional condition afforded. These observations lend support to the hypothesis suggested by the transfusion experiments that an improved flow of blood augments the rate of the uterine contractions.

We may conclude from the experiments on hemorrhage and transfusion (summarized in table 4) that the changes in the tone of the uterus described in connection with osmotic changes in the blood have nothing to do with increase or decrease of blood volume.
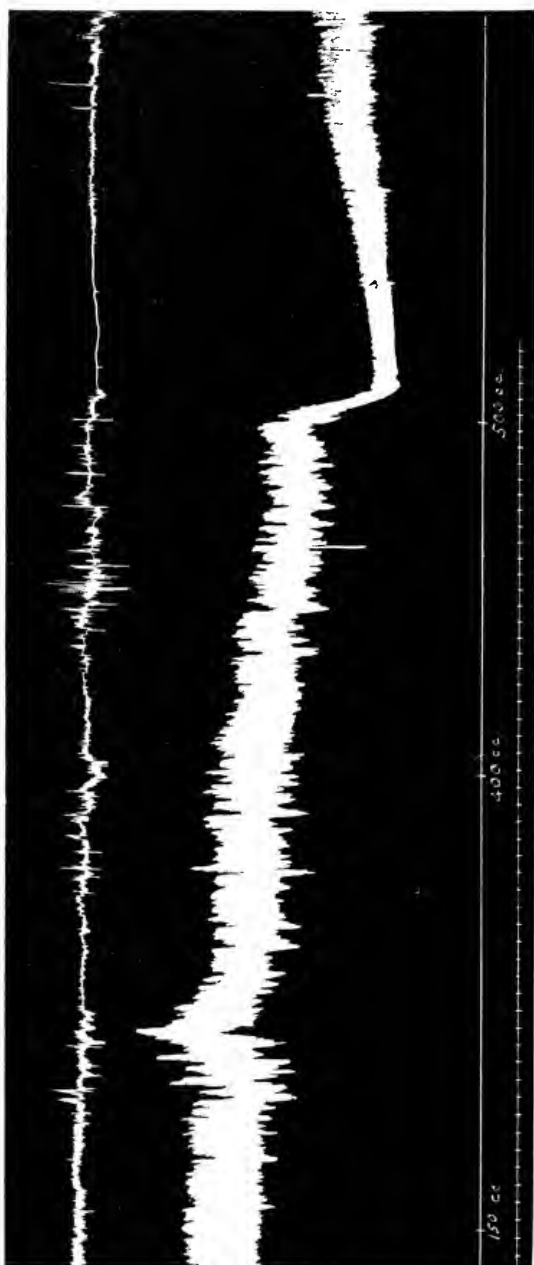
Fig. 6. Dog 1

Effects of three hemorrhages as indicated by marks on third line, 150, 400 and 500 cc. respectively

3. THE ACTION OF ACID AND OF ALKALI

If the experiments illustrating the changes in the tone of the uterus following injections of hypo- and hypertonic salt solutions have been correctly interpreted, we may assume that any circumstance which causes the organ in vivo to take up water will tend to increase its tone. Conversely, loss of water should diminish the tone.

On the supposition that an increase in the hydrogen-ion concentration would promote inbibition of water by the uterus and a decrease (not too great) in the hydrogen-ion concentration exhibit an opposite effect, it was believed that the addition of
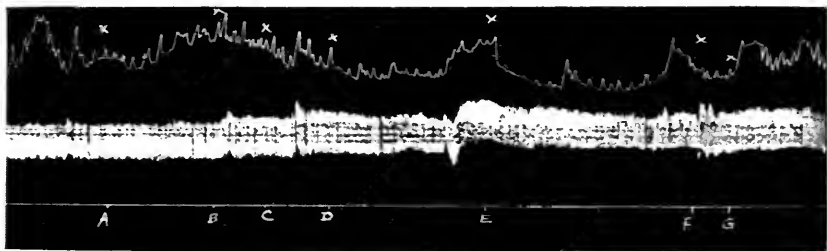
Fig. 7. Dog 14

Intravenous injections: A, 5 cc. 0.9 per cent NaCl; B, 5 cc. 1 per cent NaHCO$_3$; C, 5 cc. 5 per cent NaHCO$_3$; D, 10 cc. 5 per cent NaHCO$_3$; E, 10 cc. 5 per cent Na$_2$CO$_3$; F, 10 cc. 1 per cent lactic acid; G, 10 cc. 5 per cent lactic acid. The time interval from A to G is one hour.

acid to the blood would tend to contract the uterus and of alkali, unless too highly concentrated, to relax it. A few preliminary experiments of this sort have been made in which this hypothesis appears confirmed. These may be illustrated by figure 7, in which the activity of the uterus of a pregnant animal is illustrated.

The first intravenous injection (A) of 5 cc. 0.9 per cent NaCl is there shown to have resulted in a gradual increase in the tone of the organ. This was followed by three successive injections of sodium bicarbonate (B, C, D)—5 cc. of 1 per cent, 5 cc. of 5 per cent, and 10 cc. of 5 per cent respectively. The consequence of the bicarbonate injections was to relax the uterus; this was

followed by a spontaneous recovery of tone together with some
increase in the blood pressure. At this point (*E*) an injection
of sodium carbonate was made. Ten cubic centimeters of a 5 per
cent solution of the stronger alkali resulted in a rapid relaxation
of the organ with temporary inhibition of the activity. After a
longer interval in which the activity and tone of the uterus
recovered, injections of lactic acid (*F, G*) were instituted, 10 cc.
of 1 per cent having no immediate effect but 10 cc. of 5 per cent

TABLE 5

Effects of altering H-ion concentration of blood

DOG NUMBER	INJECTION		UTERINE CONTRACTIONS			BLOOD PRESSURE
	Substance	Amount	Tone	Amplitude	Rate	
		cc.				
13	5 per cent HCl	10	++	0	0	0
17 P	5 per cent HCl	2	+	0	0	
14 P	1 per cent HCl	10	0	0	0	0
14 P	10 per cent $CH_3CHOH \cdot COOH$	10	0	0	0	0
13	10 per cent $CH_3CHOH \cdot COOH$	10	++	−	−	(− −)
14 P	5 per cent $CH_3CHOH \cdot COOH$	10	++	−	−	−?
14 P	1 per cent $CH_3CHOH \cdot COOH$	10	0	0	0	0
14 P	1 per cent $NaHCO_3$	5	0	0	0	0
13	5 per cent $NaHCO_3$	5	(−)	0	0	0
13	5 per cent $NaHCO_3$	10	(−)	0	0	0
14 P	5 per cent Na_2CO_3	10	(− −)	(− −)	(− −)	0
13	5 per cent NaOH	10	(− −)	0	0	(− − −)
17 P	5 per cent NaOH	2	(− −)	0	0	(− −)
17 P	5 per cent NaOH	2	(−)	0	0	(−)

producing a permanent increase in the tone with a preliminary
inhibition of activity.

In the same animal it was later found that a 10 cc. injection
of 10 per cent lactic acid was without further effect. Recourse
was then had to a stronger acid; while 10 cc. of 1 per cent hydro-
chloric acid yielded no result the subsequent introduction of
10 cc. of 5 per cent hydrochloric acid produced a marked contrac-
tion of the uterus. Later, with the re-establishment of normal
conditions, the organ relaxed to its former level.

From the above and other experiments (table 5) it is concluded that, within certain limits, the tone of the uterus tends to vary in the same direction as the hydrogen-ion concentration of the blood.

CLINICAL SUGGESTIONS

Further work along the lines pursued in these investigations may lead to practical applications. For example, in conditions exhibiting atony of the uterus, intravenous injections of hypotonic saline might prove beneficial. Similarly it may be found possible with hypertonic saline to relieve uterine spasm. Where the conditions do not demand haste the same results might be accomplished by the employment of similar measures by the alimentary route. Distilled water or weak saline solutions could be given by stomach or rectum to increase uterine tone, or similar treatment with the cathartic salts might relax the uterus.

The probability that transfusion increases the rhythmic activity lends special support to its employment in cases of severe hemorrhage with atonic uterus. Since, however, the introduction of whole blood does not increase the tone it is possible that the ideal treatment would be some form of transfusion with diluted (hypotonic) blood.

SUMMARY

Experiments made upon the intact uterus of dogs anesthetized with morphine and chloretone have yielded the following results:

1. The tone of the uterus was found to vary inversely with the salt concentration of the blood when altered by intravenous injections.

2. The above changes were found to be independent of the blood pressure for hypo- and isotonic solutions sometimes raised and sometimes lowered the blood pressure while the effect on uterine tone was constant. Although hypertonic sodium chlorid always reduced the blood pressure simultaneously with the relaxation of the uterus the former recovered independently of the latter, and 14 per cent acacia injections which increased

the blood pressure were none the less efficient in diminishing uterine tone.

3. That the effects of saline injections were not due to changes in the blood volume is shown by the transfusion as well as the hemorrhage experiments, for the former affected the tone of the uterus differently in each case and the latter was without effect upon it.

4. Isotonic sodium chlorid injections occasionally increased the uterine tone; also when 3 or 7 per cent acacia was added. These effects were always associated with some rise of blood pressure, a phenomenon which does not necessarily affect the uterine tone.

5. Injection of whole blood (transfusion) increased the rate of uterine contractions, while loss of blood abolished them.

6. When acids or alkalies were injected the tone of the uterus varied in the same direction as the hydrogen-ion concentration of the blood.

7. A number of clinical applications of the above work have been suggested.

CONCLUSION

After intravenous injections the tone of the uterus is especially affected by those changes in the blood which would promote alteration in the fluid content of the uterine muscle.

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THE MODE OF ACTION OF POTASSIUM UPON ISOLATED ORGANS

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Zwaardemaker and his co-workers (27, 29) during the past few years have published a large number of papers in support of the view that the presence of potassium is essential for the maintainance of the function of isolated tissues because of its radioactivity, and they have claimed that potassium can be replaced in Ringer's fluid by other radio-active substances in aequiradioactive quantities.

The most important observation which supports this view is that when an isolated frog's ventricle is arrested by lack of potassium, the introduction of small traces of radio-active substances will cause it to resume an automatic beat.

Zwaardemaker (21) has put forward the following conclusion:

In a large number of systems the potassium atom may, as regards function, be replaced by all the other radio-active elements, the heavy ones as well as the light ones, provided that the doses are equi-radioactive . . . . thus a radio-active element, whichsoever it may be, added in a dosage equi-radio-active to potassium, to a potassium-free Ringer's mixture, renders such a solution as effectual a circulating fluid for the heart of a cold blooded animal as the original fluid.

These conclusions, which indicate that the special function of potassium in the body is due to its radioactivy, are obviously of great and fundamental importance in our general conception of the dynamics of living matter.

The writer (4) made experiments with isolated frog's heart, and studied the changes which occurred when potassium-free Ringer was substituted for normal Ringer, comparing with these

the effects of substituting normal Ringer by Ringer minus potassium but with radio-active substances added. The conclusion drawn was:

I consider therefore that although uranium will excite a heart arrested from lack of potassium, yet it cannot be said to replace potassium in the manner in which rubidium will replace potassium.

Zwaardemaker (28) has replied to this observation, stating that my failure to obtain positive results with uranium was due to my using too high a concentration of potassium in my normal Ringer.

If other radio-active substances are true substitutes for potassium this substitution effect should be manifested over a wide range of conditions, and these experiments of mine were intended to determine whether this substitution effect occurred under any other conditions than the special ones employed by Zwaardemaker.

The present experiments represent a further attempt to determine to what extent potassium can be replaced by rubidium and caesium and by the heavy radio-active metals uranium and thorium, in solutions which are used to preserve the activity of isolated tissues of frogs and mammals.

The chief questions are (1) what concentration of potassium is to be considered normal, (2) what are the approximate radio-active equivalents of potassium, rubidium, caesium, uranium and thorium, (3) what are the effects of altering the concentration of the potassium, (4) to what extent will addition of radio-active substances compensate for partial or complete lack of potassium, and (5) whether there is any resemblance between the effects produced by excess of potassium and those produced by excess of radio-active metals.

Zwaardemaker claims that the action of potassium is due to its radio-activity, and, if this true, evidence that potassium can be replaced by uranium and thorium should be obtained over a wide range of conditions.

The evidence put forward in this paper shows that rubidium acts as a true substitute for potassium, and that it can replace

potassium in all tissues and that excess of rubidium acts like excess of potassium; on the other hand, uranium and thorium can compensate to a certain extent for lack of potassium in the frog heart under a certain narrow range of conditions, but under all other conditions in the frog's heart, and under all conditions in other tissues there is no evidence at all that uranium and thorium act as substitutes for potassium.

EXPERIMENTS UPON FROGS' HEARTS

Isolated frogs' hearts were perfused with two cannulae, one in the sinus and the other in the aorta; other experiments were made with Kronecker's cannulae, and with isolated strips of the heart suspended in Ringer. I have been much impressed with the superiority of the results obtained when fluid is passed into the ventricle from the auricle, over those obtained with Kronecker's cannula; with the former method the heart appears to be much more thoroughly washed out than it is with other methods and the response to changes in the ionic content of Ringer is more rapid and stronger when complete hearts are used than when the ventricle alone is used.

The Ringer used was of the following composition NaCl 0.65 per cent; CaCl$_2$ anhydrous 0.012 per cent; KCl 0.015; NaHCO$_3$ 0.016 per cent pH 7.7. I consider this to be a normal Ringer because it corresponds to what is known of the composition of frog's serum, and it maintains the functions of the frog's heart for prolonged periods in a state which resembles the functions of the heart in the intact frog.

Urano (25 and 26) obtained the following analysis for frog's serum. NaCl 0.56 per cent; CaCl$_2$ 0.025 per cent; his estimates of KCl unfortunately varied from 0.004 to 0.08 per cent. The writer found that the frog's serum contained 0.017 per cent KCl

The power of such a Ringer to maintain the normal functions of the frog's heart is shown in table 1, in which the heart perfused with this Ringer is demonstrated to differ little from the normal heart in rate of beat, conduction and duration of electrical and mechanical response.

The effect upon the isolated frog's heart of alterations in the potassium content of Ringer has been described in detail in previous papers (Clark (3), Daly and Clark (7)). The reduction of potassium chloride to one quarter normal, that is, to 0.004 per cent produces in a few minutes well marked effects, the chief of which are a prolongation of the a-v interval, a prolongation of the duration of the mechanical response, and imperfect relaxation in diastole.

TABLE 1

	RATE PER MINUTE	P-R INTERVAL	A-V INTERVAL	DURATION OF ELECTRICAL RESPONSE	DURATION OF MECHANICAL RESPONSE
		seconds	seconds	seconds	seconds
Frog's heart in situ with normal circulation......................	33	0.38	0.4	0.85	0.95
Heart perfused for thirty minutes with normal Ringer..............	33	0.34	0.35	1.00	1.00
Heart perfused for twenty-four hours with normal Ringer........	18		0.6		0.9

TABLE 2

Isolated frog's ventricle stimulated every fifteen seconds

	LENGTH OF MECHANICAL RESPONSE	LENGTH OF REFRACTORY PERIOD
	seconds	seconds
Normal Ringer (KCl 0.016 per cent)	1.0	0.9
Ventricle perfused with Ringer containing KCl 0.004 per cent		
After two minutes......................................	1.7	1.2
After twenty minutes.................................	1.5	1.4

The typical effects produced by reduction of the potassium content are shown in table 2. In this experiment the frog's ventricle was perfused with a Kronecker's cannula, the frequency of contraction was kept regular by stimulating the ventricle every fifteen seconds, and the refractory period was estimated by putting in second stimulations at irregular intervals. The table shows clearly that the reduction of the potassium chloride content of the Ringer to 0.004 per cent produces a wholly abnormal type of beat.

Zwaardemaker has taken as his test for the normality of his perfusion fluid its power of maintaining spontaneous beats in the isolated ventricle, and by this means finds (28) that the optimal KCl content of Ringer in summer may be as low as 0.0005 per cent.

This test I do not consider a valid one, for a Ringer of such composition will never maintain a beat on the intact heart which in any way resembles the normal beat.
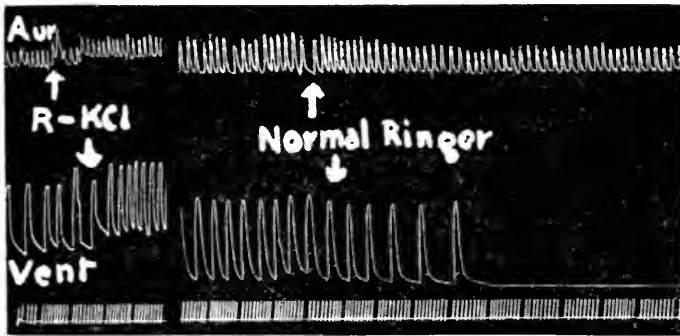
FIG. 1 THE EFFECT OF ALTERATIONS OF THE POTASSIUM CONTENT UPON
SEPARATE STRIPS OF FROG'S AURICLE AND VENTRICLE
SUSPENDED IN RINGER

The change from normal Ringer to Ringer minus KCl does not alter the auricular rate but increases the ventricular rate from 12 to 24 per minute. The change from Ringer minus KCl to normal Ringer increases the auricular rate from 24 to 28 per minute but produces arrest of the ventricle.

The optimal ionic content for maintaining the automaticity of the ventricle is quite different from that which best maintains a normal beat in the whole heart.

Sakai (22) showed that the isolated frog's ventricle maintained its automatic rhythm better when the NaCl content was reduced to 0.1 per cent than it did with normal Ringer.

Kolm and Pick (14) used strips of frog's ventricle and auricle and showed that the change from normal Ringer to Ringer minus potassium depressed the contractions of the auricular strips but stimulated the ventricular strips, and that the change from potassium free to normal Ringer produced the reverse effects.

I confirmed these observations and this effect is shown in figure 1. This figure shows that fluids which best maintain the automaticity of the ventricle are not necessarily of a composition suitable for maintaining the functions of the rest of the heart.

I consider that the only way to test whether a fluid has a potassium content which is in equilibrium with the heart cells is to determine whether the fluid can maintain in the whole heart a beat of a character similar to that observed in the heart of an intact frog.

This test shows that only a Ringer's fluid with a KCl content between 0.01 and 0.02 per cent is normal; that is, provided that the $CaCl_2$ content is 0.01 per cent, for, since calcium and potassium act as antagonists, an alteration in the KCl content can be neutralised within certain limits by a corresponding alteration in the $CaCl_2$ content.

Spontaneous beats of the isolated ventricle can be maintained for prolonged periods by fluids of very varying composition, and occur more readily when the KCl content is reduced below 0.004 per cent than they do in normal Ringer, but I do not consider that this is evidence that such fluids are to be regarded as normal perfusion fluids.

AEQUI-RADIO-ACTIVITY

The estimation of the aequi-radio-concentrations of different elements when perfused through the frog's heart is a matter of great difficulty.

The chief evidence available is summarized in table 3, and from this it appears that the activity of the beta rays of U, Rb, and K is about 100-1000:10:1. These ratios are very uncertain and they leave out of account the alpha rays of uranium, to which over 99 per cent of the chemical actions produced by its radiations are due. Moreover all work upon the action of radium emanation upon living tissues has shown that the alpha rays have a far stronger action upon living tissues which they reach, than have the beta rays.

The average range of the rays through tissues appears to be for alpha rays about 20μ and for beta rays 2 mm. (Colwell and Russ (6)). Both the alpha and beta rays from a radio-active solution perfused through a frog's heart will therefore reach the heart cells.

Zwaardemaker claims that the alpha and beta rays have antagonistic actions, and if this is correct any attempt to find any biological radio-active equivalents for potassium and uranium

TABLE 3

ELEMENT	CHARACTER OF RADIATIONS	RADIO-ACTIVE EQUIVALENTS
1. Uranium salts	Alpha, beta and gamma rays	Nearly the whole of the ionising power is due to the alpha rays. (Rutherford (21).)
2. Thorium salts	Alpha, beta and gamma rays.	Ionising power about equal to that of uranium. Beta rays weaker than those of uranium. Rutherford.
3. Rubidium salts	Beta rays only, these rays are less penetrating than those of potassium. No alpha rays.	Radio-activity about $\frac{1}{15}$ that of beta rays of uranium, and $\frac{1}{10}$ that of beta rays of thorium (Hahn and Rothenbach (11).)
4. Potassium	Beta rays only, these rays are about as penetrating as the beta rays of uranium.	Radio-activity $\frac{1}{4}$ that of rubidium (Campbell 29)) and $\frac{1}{1000}$ that of the beta rays of uranium. (Rutherford.)
5. Caesium	No evidence of radio-activity has been found, in spite of careful investigation	

is of course hopeless, since the former sends out only beta rays, and nearly the whole of the radio-activity of the latter is due to the alpha rays.

Redfield and Bright (20) have shown however that soft beta rays have a stronger biological action than hard beta rays, and since the radio-activity of rubidium is at least seven times as great as that of potassium and its rays are softer, therefore rubidium should be at least ten times as active as potassium when tested by perfusion through the frog's heart.

A. J. CLARK

THE POWER OF URANIUM AND THORIUM TO REPLACE POTASSIUM IN THE FROG'S HEART

The removal of the potassium from Ringer produces in the isolated frog's heart within five minutes certain very definite alterations in the form of the beat, and the heart is arrested in systole in about thirty minutes. The addition of uranium or thorium to potassium-free Ringer does not influence in any demonstrable manner the effects produced upon a fresh heart by lack of potassium. These effects are shown in table 4, and have also been described in a previous paper (4).

TABLE 4

TIME	RATE PER MINUTE	LENGTH OF A-V INTERVAL	LENGTH OF MECHANICAL RESPONSE
minutes		*seconds*	*seconds*
I 0	36	0.37	1.0
	Ringer minus potassium perfused		
5	26	0.56	1.9
30	24	0.65	2.0
35	Heart arrested in systole		
40	Heart relaxed into diastole, but remained arrested		
II 0	40	0.25	0.9
	Ringer minus potassium plus uranium nitrate 0.0025 per cent perfused		
5	28	0.7	3.8
20	12	1.0	4.3
30	Heart arrested in systole		
40	Heart relaxed into diastole and remained arrested		

On the other hand if potassium is replaced by a chemically equivalent amount of rubidium no alteration in the beat can be detected, and the heart will continue a perfectly normal beat for several hours. Caesium will only partially replace potassium, but only when caesium is substituted for potassium the heart will continue a beat for many hours. These effects are shown in figure 2.

An excess of potassium produces a marked effect upon the frog's heart in a few seconds. An increase of the KCl content on Ringer from 0.015 to 0.064 per cent produces in ten minutes

the following effects: reduction of the height of contraction to one third, and an increase of the a-v interval to more than double the normal length.
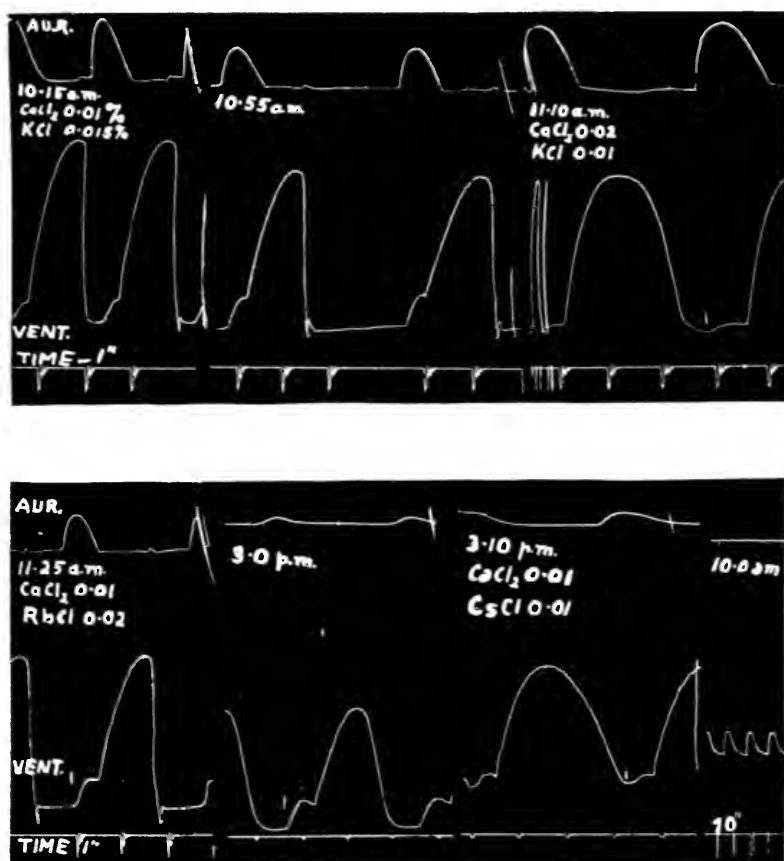
FIG. 2. ISOLATED FROG'S HEART PERFUSED, MOVEMENTS OF AURICLE AND VENTRICLE RECORD

The heart maintains a normal beat with a Ringer containing $CaCl_2$ 0.01 per cent and KCl 0.015 per cent: the alteration to $CaCl_2$ 0.02 and KCl 0.01 per cent produces at once an increase in the duration of the mechanical response. The substitution of rubidium for potassium maintains a normal beat. The substitution of caesium for potassium produces an abnormal beat but the automatic activity of the heart is maintained for many hours.

The addition of 0.01 per cent urarium nitrate to normal Ringer produces no certain effects within an hour. A source of error must however be mentioned: the salts of uranium and thorium are strongly acid and are precipitated in neutral solution. It is therefore necessary to neutralise the uranium salt as far as possible before adding it to the Ringer and then to test the reaction of the Ringer carefully after it has been added.

These results show that rubidium and caesium will replace potassium in Ringer's fluid, as was stated by Ringer in 1884. Rubidium replaces potassium in aequimolecular amounts, but the radioactivity of the hot rubidium band is nearly ten times that of potassium, and therefore, if rubidium acted as a substitute for potassium in virtue of its radio-activity, the addition of 0.02 per cent RbCl should produce the same effects as the addition of 0.125 per cent KCl; this amount of KCl would produce diastolic arrest of the heart in a few minutes, whereas the concentration of RbCl mentioned maintains a normal beat for hours. Caesium, which has no radio-activity according to all of the recognised physical tests, can maintain the activity of the frog's heart for many hours.

These facts support the view that the action of potassium, rubidium, and caesium on the heart is a chemical one and has nothing to do with the radio-activity of these elements.

THE ACTION OF RADIO-ACTIVE SUBSTANCES UPON THE FROG'S
HEART WHEN PARTIALLY DEPRIVED OF POTASSIUM

Zwaardemaker has shown that when the frog's ventricle is arrested by lack of potassium or when it is perfused with a fluid containing an abnormally low potassium content, then the change to a fluid free from potassium, containing a radio-active substance, will induce or maintain a regular atomatic rhythm.

I have performed experiments under these conditions and have obtained positive results, although I have found that the results are extraordinarily variable, and are difficult to bring off with certainty.

I concluded from my experiments that if a frog's heart was reduced to a certain stage of potassium lack, and was then

perfused with a radio-active substance, an idio-ventricular rhythm might be induced, and this rhythm might last for several hours.

The exact point of potassium lack required to obtain this effect was very difficult to attain and the results were nearly always negative with frogs in poor condition.
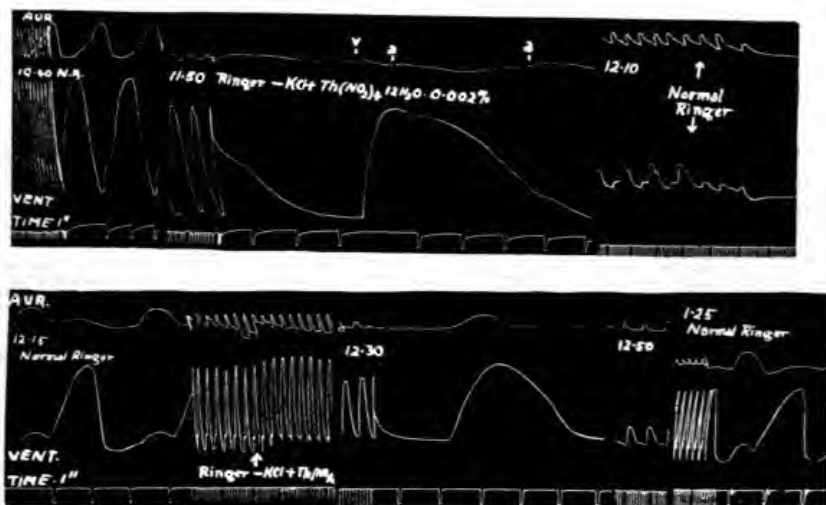
FIG. 3. THE ACTION OF THORIUM UPON THE FROG'S HEART

The following changes in the perfusion fluid were made.   10.40 perfused with normal Ringer; 10.55 perfused with KCl free Ringer, arrest of heart at 11.35; 11.37 perfused with KCl free Ringer containing thorium nitrate 0.002 per cent, spontaneous beats commenced at 11.47 and continued until 12.10 when normal Ringer was perfused; this caused temporary arrest of the heart but a normal beat commenced at 12.14; at 12.15 Ringer minus KCl plus thorium nitrate induced idioventricular beats which continued until 1.10 when the heart ceased to contract; perfusion with normal Ringer finally restored a normal beat.

Figure 3 shows a typical positive result.   The heart was stopped by lack of potassium and the introduction of thorium started a beat which was of normal amplitude, but idioventricular; moreover the duration of the mechanical response was about three times as long as normal.   Later on an auricular rhythm developed but the a-v interval was about ten times the

normal length; the addition of potassium stopped these contrac-
tions but a normal rhythm developed after a few minutes; as
soon as this rhythm had developed Ringer minus potassium plus
thorium was perfused and again started an idioventricular
rhythm, which continued for nearly an hour.

The point which I wish to emphasize is that the beat obtained
with the thorium was of normal amplitude, and if it had been
recorded on a sufficiently slowly moving drum might have ap-
peared a normal beat; the fast moving record shows its abnormal
character however.
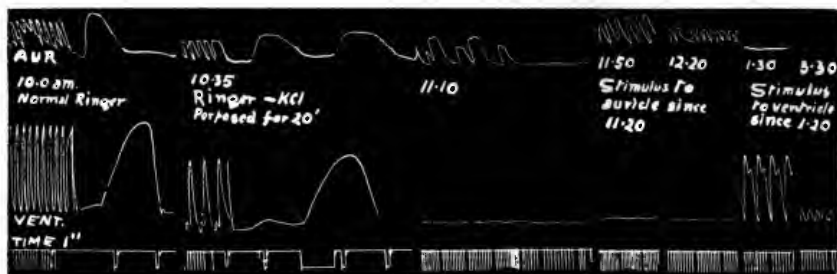
FIG. 4. FROG'S HEART PERFUSED WITH POTASSIUM-FREE RINGER FROM 10.15
ONWARDS

Spontaneous beats ceased at 11.10. Induction shocks (26 per minute) applied
to auricle produced regular response for two hours: after this induction shocks
applied to the ventricle produced ventricular response for two hours more.

Uranium and thorium appear to irritate the isolated frog's
heart and thus stimulate activity in the heart after it has been
arrested by lack of potassium. A very similar effect can how-
ever be produced by stimulating a heart by electric shocks after
it has been arrested by lack of potassium. This effect is shown
in figure 4. In this case perfusion with potassium-free Ringer
stopped the spontaneous beats at 11:10 but stimuli to the auricle
produced regular responses for two hours and when the auricle
became irresponsive stimulation of the ventricle produced con-
tractions a further two hours. This shows very clearly that the
heart retains the power to respond to stimuli long after it has
lost the power of initiating spontaneous beats.

THE WASHING OUT OF POTASSIUM FROM THE HEART

A series of experiments were made to determine what effect perfusion with potassium-free Ringer had upon the potassium content of the frog's heart.[1]

The potassium was estimated by the technique described by Kramer (15). The results are shown in table 5.

The analysis of the fresh hearts shows that these contain about 0.2 per cent potassium, and that two hours perfusion with K-free Ringer reduces this to 0.13 per cent, and that after six hours perfusion the K content is reduced to 0.1 per cent or about one-half of the original total.

These results agree with those of Urano (25), who found that soaking thin muscles of the frog in isotonic sugar for 22 hours reduced the potassium content from 0.32 to 0.23 per cent.

This suggests some curious problems; the fresh muscle contains about 0.4 per cent KCl, and Ringer contains only 0.015 per cent. The beta rays of potassium are exceptionally penetrating and less than one-half would be absorbed by the whole thickness of the heart wall (Colwell and Ross (6) found that 15 per cent of hard beta rays of radium were absorbed by 0.65 mm. of tissue). It is really impossible to imagine how potassium outside the heart cells could exert any different radio-active effect upon the cells than does the potassium inside the cells. The function of the cells is however profoundly altered in less than five minutes by a reduction of the potassium in the perfusion fluid from 0.015 to 0.004 per cent, and perfusion with fluid containing no potassium causes arrest of the heart in about thirty minutes, although the cells still contain more than two thirds of their original potassium.

All of these results are perfectly easy to explain if it is assumed that potassium exerts some physico-chemical action upon the surfaces of the cells, but they seem impossible to explain if potassium is supposed to act in virtue of its radio-activity.

[1] Mitchell and Wilson (Jour. Gen. Physiol. iv, 45, 1921) studied the effect of perfusing frogs legs with potassium free Ringer, and found that 18 hours perfusion only produced a loss of about 10 per cent of the potassium in the muscles.

TABLE 5

DATE	NUMBER OF HEARTS	WEIGHT OF HEARTS WHEN FRESH	POTASSIUM IN HEARTS	POTASSIUM PRESENT IN FRESH HEARTS
1. Hearts not perfused				
		grams	*mgm.*	*per cent*
March 22, 1921..............	5	0.300	0.635	0.21
March 23, 1921..............	5	0.315	0.505	0.16
March 24, 1921..............	6	0.390	0.827	0.21
April 4, 1921................	4	0.240	0.501	0.205
Average				0.196
2. Hearts perfused for two hours with Ringer minus potassium				
March 22, 1921..............	5	0.370	0.575	0.155
March 23, 1921..............	4	0.390	0.500	0.13
April 7, 1921................	4	0.230	0.230	0.10
Average				0.13
3. Hearts perfused for six hours with Ringer minus potassium				
March 23, 1921..............	4	0.330	0.310	0.094
March 23, 1921..............	4	0.250	0.320	0.128
April 7, 1921................				
Average				0.111

4. The amount of potassium washed out of the hearts during perfusion

DATE	NUMBER OF HEARTS	LENGTH OF PERFUSION WITH RINGER MINUS POTASSIUM	POTASSIUM FOUND IN PERFUSION FLUID	POTASSIUM FOUND IN HEARTS
		hours	*mgm.*	*mgm.*
March 23, 1921..............	4	2	0.36	0.50
March 23, 1921..............	4	6	0.41	0.31
April 7, 1921................	4	6	0.24	0.32

THE REPLACEMENT OF POTASSIUM BY RADIOACTIVE METALS IN LOWER ORGANISMS

Peters (19) found that potassium could not be replaced by uranium in culture media for protozoa.

Loeb (16) found that the eggs of Arbacia would not develop in media in which potassium was replaced by uranium or thorium.

These experiments show that potassium cannot be replaced by radio-active substances in these two cases, and this suggests that the apparent replacement obtained in the frog's heart is a special phenomenon, and not an example of a general rule.

THE REPLACEMENT OF POTASSIUM BY URANIUM IN THE RABBIT'S HEART

Janninck and Feenstra (13) found that when the isolated rabbit's heart was perfused with potassium-free Ringer it was arrested in a few minutes, but that if the heart was immediately
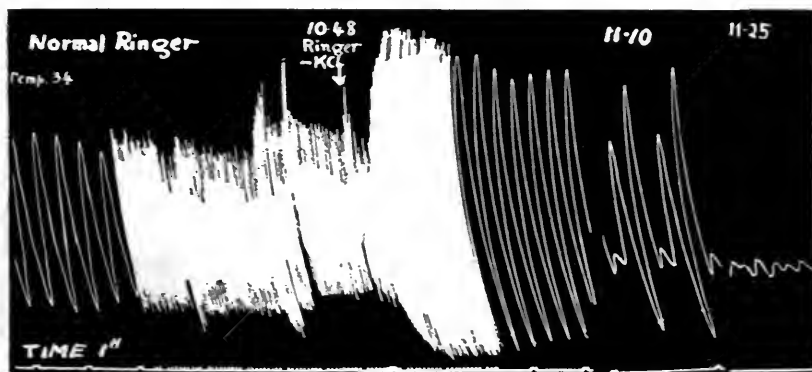
FIG. 5. ISOLATED AURICLE OF RABBIT SUSPENDED IN RINGER

Potassium free Ringer produced an initial increase in rate and amplitude of contraction, followed by a decrease in the force of contraction and arrest in partial systole in about forty minutes.

perfused with a fluid containing uranium nitrate 0.003 per cent a beat recommenced.

I determined the action of lack of potassium upon rabbit's auricles. The auricles were cut off from the ventricles and suspended in Ringer, through which a free stream of oxygen was passed.

The Ringer contained 0.042 per cent KCl and increasing the KCl content fourfold (to 0.17 per cent) produced an immediate depressant effect upon the auricle, the amplitude of the beat

being reduced to one quarter in a few minutes. The addition of uranium nitrate 0.005 per cent produced no effect upon the auricle in twenty minutes (fig. 6).

Deprivation of potassium produced an initial stimulant effect and both the force and rate of beat were increased. This was followed in a few minutes by a depressant action and the auricle ceased to beat in about forty minutes (fig. 5). These results are quite different from those noted by Janninck and Feenstra upon the whole heart.

The substitution of Ringer minus potassium plus uranium or thorium produces almost the same results as does Ringer minus potassium (fig. 6).
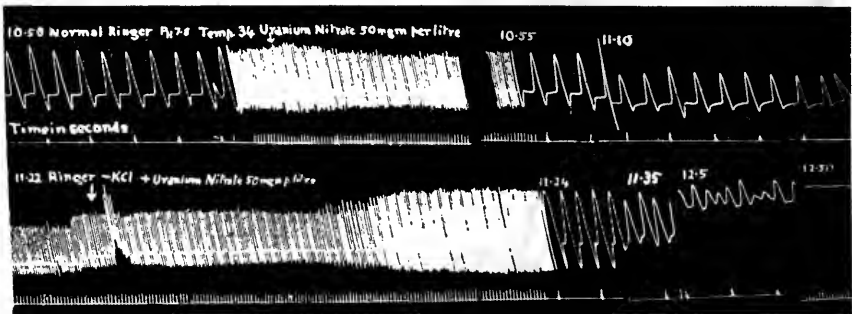
FIG. 6. THE ACTION OF URANIUM UPON THE ISOLATED AURICLE OF THE RABBIT

Uranium nitrate 0.005 per cent added to normal Ringer produced no effect in thirty minutes. The introduction of Ringer minus KCl plus uranium nitrate 0.005 per cent produced the same effects as did Ringer minus KCl.

The addition of potassium to the auricle when it is in the excitement stage following upon deprivation of potassium produces an immediate sedative effect, and the beats become smaller and more regular. This effect is shown in figure 7, curve 1; rubidium has a similar action but caesium has none, and thorium also has no certain effect, and with the thorium the auricle died in systole in about thirty minutes. These experiments show that in the rabbit's heart potassium can be replaced by rubidium, but not by caesium, thorium, or uranium.
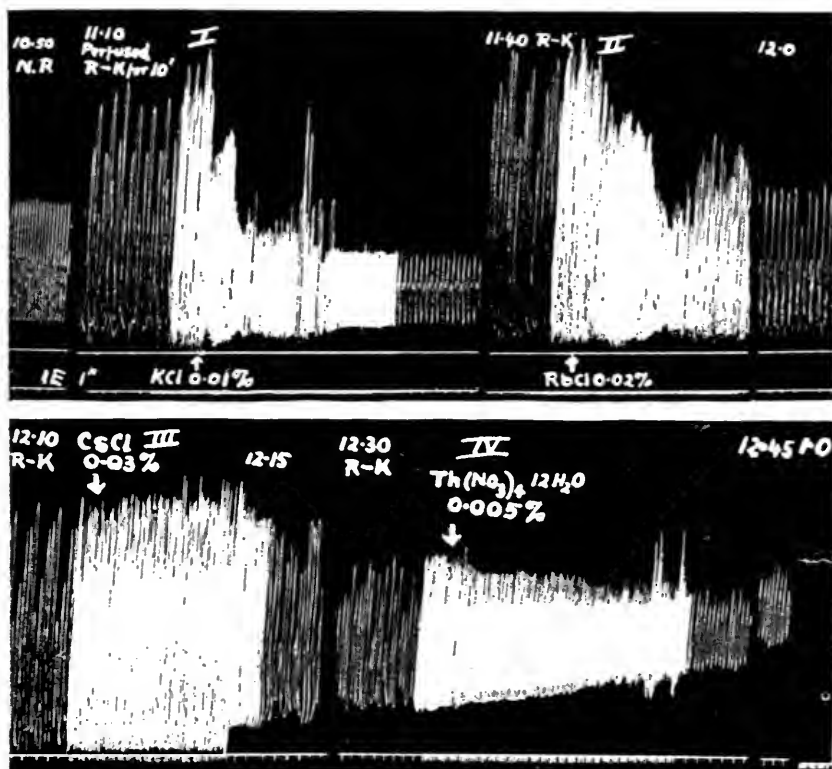
FIG. 7. THE ACTION OF POTASSIUM, RUBIDIUM, CAESIUM AND THORIUM UPON
THE ISOLATED RABBIT'S AURICLE WHEN THIS IS EXCITED BY
LACK OF POTASSIUM

The auricle was excited in each case by deprivation of potassium for a few minutes.

I. 0.01 per cent KCl restored a regular beat in a few minutes.

II. 0.02 per cent RbCl produced the same effect as potassium.

III. 0.03 per cent CsCl produced no certain effect.

IV. 0.005 per cent thorium nitrate produced no certain effect and the heart auricle died in systole in thirty minutes.

THE ACTION OF POTASSIUM UPON PLAIN MUSCLE

A moderate excess of potassium causes contraction of all plain muscle. This has been shown to be true for the following preparations: the frog's stomach (Fruboese 19); the hen's oesophagus (Fienza 8); the cat's oesophagus and the uterus of the cat and guinea-pig (Mathison 17); the bronchial muscle of sheep (Trendelenburg (24)).
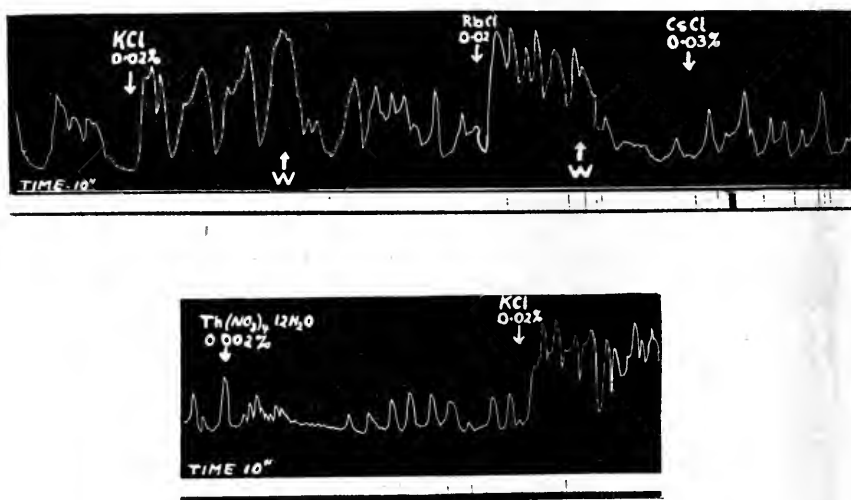
FIG. 8. THE ACTION OF POTASSIUM, RUBIDIUM, CAESIUM AND THORIUM IN PRODUCING CONTRACTIONS OF THE ISOLATED UTERUS OF THE RABBIT

Hanke and Koessler (12) found that the addition to Ringer of 0.05 per cent KCl produced a submaximal contraction in the isolated guinea-pig's uterus, and that an equal effect was produced by 0.01 per cent RbCl and by 0.15 per cent CsCl. They concluded that rubidium was about 8 times as active as potassium, and that caesium was much less active.

Soref (23) found that rubidium, uranium, and thorium all caused contractions of the isolated uterus of the guinea pig in lower concentrations than did potassium, but that caesium was less active than potassium.

Using the isolated uterus of the rabbit, the writer found that a distinct increase of tonus was produced by the addition of 0.02 per cent (0.0026N) KCl to the normal Ringer, which already contained 0.042 per cent (0.0057N) KCl, and that the addition of 0.02 per cent (0.0017N) RbCl produced a greater effect, while the addition of 0.02 per cent (0.0012N) CsCl produced very little effect (fig. 8). The relative action of these three substances upon the isolated uterus of the kitten is shown in figure 9.

Caesium undoubtedly has only about half the action of potassium and rubidium has a stronger action than potassium, but figure 9 shows that the ratio between the activities of Rb and K is nearer 2:1 than 8:1. Thorium and uranium in all cases failed to produce any action upon the isolated uterus, provided that care
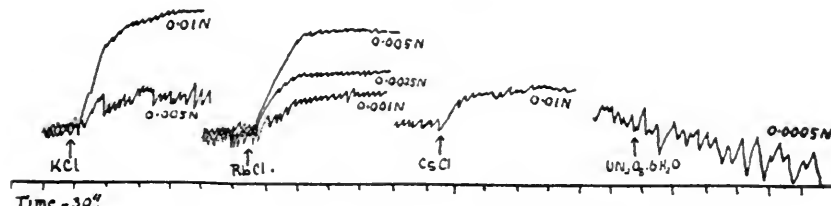
FIG. 9. THE ACTION OF POTASSIUM, RUBIDIUM, CAESIUM AND THORIUM IN PRODUCING CONTRACTIONS IN THE ISOLATED UTERUS OF A KITTEN

was taken to introduce neutralised solutions. Moderate concentrations of these salts when added without any precautions to obtain neutrality produced an acidity strong enough to produce a contraction of the isolated guinea-pig's uterus.

THE EFFECT OF POTASSIUM UPON PLAIN MUSCLE

Lack of potassium was found to produce contraction of all the plain muscle examined.

Excess of potassium and lack of potassium both cause vasoconstriction in the perfused frog (fig. 10).

Lack of potassium produces contraction of the isolated uterus of the rabbit (figs. 11 and 12) and this contraction is at once inhibited by the addition of a small amount of potassium. Lack
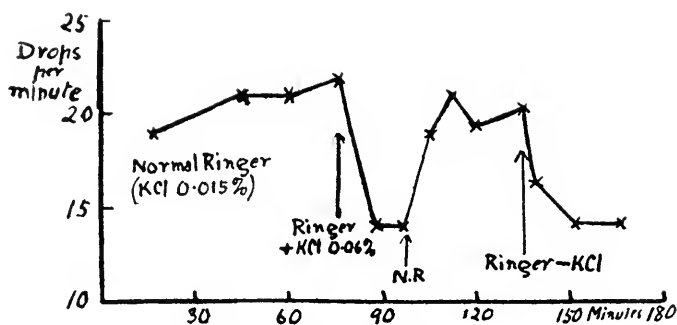
FIG. 10. THE EFFECT OF ALTERATION OF THE POTASSIUM CONTENT OF RINGER
UPON THE VESSELS OF A FROG PERFUSED THROUGH THE AORTA
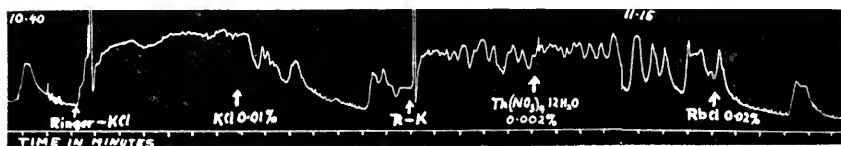
FIG. 11. THE ACTION OF POTASSIUM, RUBIDIUM AND THORIUM IN INHIBITING
THE HYPERACTIVITY OF THE RABBIT'S UTERUS PRODUCED BY
LACK OF POTASSIUM
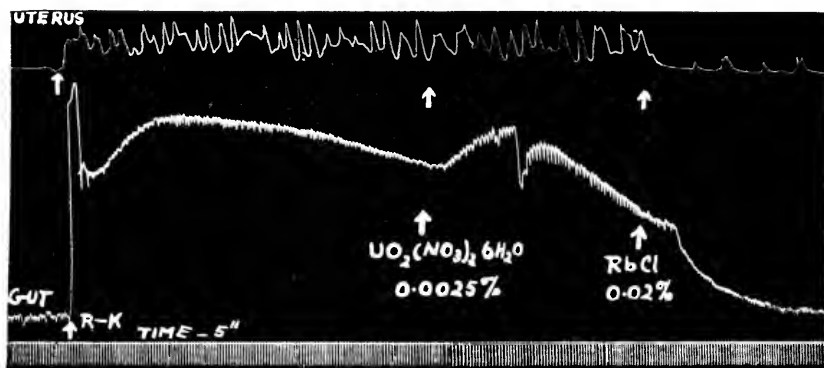
FIG. 12. THE ACTION OF URANIUM AND RUBIDIUM IN INHIBITING THE HYPER-
ACTIVITY PRODUCED BY LACK OF POTASSIUM IN THE ISOLATED
GUT AND UTERUS OF THE RABBIT
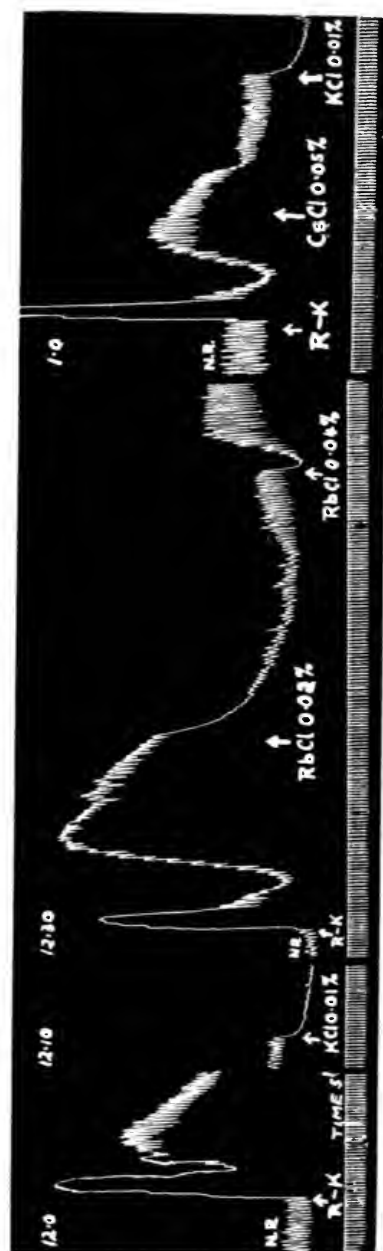
FIG. 13.   THE ACTION OF POTASSIUM, RUBIDIUM AND CAESIUM IN INHIBITING THE HYPERACTIVITY PRODUCED BY LACK OF POTASSIUM IN THE ISOLATED GUT OF THE RABBIT

of potassium also produces a prolonged spasmodic contraction in the isolated rabbit's gut and here also the addition of a small amount of potassium causes immediate relaxation (figs. 12 and 13).

Rubidium has the same effect as potassium in inhibiting the contraction produced by lack of potassium in the isolated gut and uterus. Caesium has a very feeble action, while uranium and thorium have no inhibitory effect whatever and, if anything, increase the activity of the gut and uterus.

THE ACTION OF POTASSIUM UPON NERVES

Overton (18) showed that potassium salts paralyse nerve trunks and that rubidium and caesium have a similar action.

TABLE 6

Action of potassium and of radio-active metals upon nerves of frog. The nerve alone was immersed in the solutions tested. The effects are shown as the distance of the secondary coil at which a break shock just produces stimulation

	TIME				
	0 minutes	20 minutes	40 minutes	60 minutes	90 minutes
KCl 0.37 per cent (average of 6 experiments)..	39	29	12	11	10
KCl 0.2 per cent (average of 3 experiments)...	36	34	18	18	18
Thorium nitrate 0.5 per cent, 2 experiments...	36	} No alteration in three hours			
Uranium nitrate 1.0 per cent, 2 experiments ...	35				

The results of Closson (5) showed that potassium acts upon the nerve trunks and also upon the nerve endings.

These authors found that a concentration of 0.2 per cent KCl or over was required to paralyse nerve-muscle preparations of the frog, but Benda (1) perfused frog's and showed that a concentration of 0.07 per cent KCl would produce paralysis when introduced in this way.

The writer found that thorium and uranium caused no paralysis of frog's nerve muscle preparations similar to that following potassium. Typical results are shown in table 6.

Greisheimer (10) has studied the effects of deprivation of potassium upon frog's nerves. These effects take a very pro-

longed period to demonstrate, but she found that rubidium would act as a substitute for potassium in maintaining the activity of frog's nerves.

1. Radio-active substances frequently induce an automatic rhythm in frog's heart, when these have been arrested by lack of potassium. These contractions are usually idioventricular, and are always abnormal in character.

2. A heart deprived of potassium shows typical alterations in its activity before the beat is arrested; the occurrence of these changes is not influenced by the addition of radio-active substances.

3. Excess of potassium produces typical effects upon the isolated frog's heart, upon nerve muscle preparations, and upon the isolated auricle, uterus, and gut of the rabbit; the addition of radio-active metals causes none of these changes.

4. Lack of potassium is followed by typical effects upon the isolated auricle of the rabbit, and upon the isolated gut and uterus of the rabbit; these effects are at once inhibited by addition of potassium; the presence of radio active metals does not prevent the occurrence of these effects, nor does the addition of radio-active metals inhibit these effects when they have been induced.

5. Potassium can be replaced by rubidium under all conditions in all of the preparations mentioned, and excess of rubidium causes effects similar to those produced by excess of potassium.

6. Caesium will replace potassium to a limited extent in the preparation, described.

7. A frog's heart perfused with normal Ringer has a concentration of 0.4 per cent KCl inside the cells and of 0.015 per cent KCl outside the cells. Quite small variations in the potassium content of the fluid outside the cells produce a marked effect upon the heart in a few minutes.

The concentration of potassium inside the cells is difficult to alter, and perfusion with potassium-free Ringer, for as long as six hours, only reduces it to about one-half. Perfusion with potassium-free Ringer arrests a heart in about forty minutes, and the

cells of the heart at the moment of arrest contain about 0.3 per cent KCl; that is, about 20 times the concentration of KCl in normal Ringer.

These facts are very difficult to explain on the assumption that potassium acts in virtue of its radio activity, for the potassium only emits beta rays and these are of a very hard type and are only reduced to half value by about 2 mm. of tissue, and therefore these rays should have much the same effect whether emitted by potassium within or without the cells.

SUMMARY

Rubidium acts as a perfect substitute to potassium in all of the isolated tissues examined; caesium acts as an imperfect substitute; thorium and uranium do not act as substitutes to potassium, but they act as irritants to the frog's heart and will induce automatic beats in hearts arrested by lack of potassium.

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ON SCOPOLAMINE-MORPHINE NARCOSIS

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In 1900 Schneiderlin (1) introduced scopolamine-morphine narcosis into medical practice. Since that time much work, clinically and experimentally has been done on the subject. One of the principal questions that occupied workers in this field has been, whether on administration of a mixture of scopolamine and morphine, the phenomenon of "potentiated" synergism (Bürgi's "Potenzierung") presents itself. According to Schneiderlin's experiments on men a potentiated synergism is really existing in this case as can easily be seen from table 1 given below, which is taken from one of Schneiderlin's publications.

According to Schneiderlin 2 mgm. of scopolamine or 60 mgm. of morphine when given alone to an adult do not produce narcotic symptoms, whereas half the dose of the first drug combined with half the dose of the second drug leads to a narcosis of more than two hours duration. This then is a case of true potentiated synergism ("Potenzierung").

The correctness of Schneiderlin's results cannot be doubted, but it seems that this synergistic action of scopolamine and morphine does not exert itself clearly in all cases where it has been tried in man, otherwise it would hardly be intelligible that so often the scopolamine-morphine combination gives unsatisfactory results in surgical cases, and that sometimes death follows after relatively small doses of scopolamine with or without morphine (viz., Müller, Narkologie).

Notwithstanding Schneiderlin's results then, the question whether scopolamine-morphine combination will as a rule produce synergism is not definitely settled, so that experimental work in this direction seemed advisable.

Some work on the subject had been done already in 1910 by Bürgi's pupil Hauckold (3).

TABLE 1

PATIENT	INJECTION		INSENSITIVE
As.	12 Dec.	2 h 15' 2 mgm. Sc........................	—
	14 Dec.	2 h 15' 4 cgm. Mo........................	—
	18 Dec.	3 h 6 cgm. Mo...........................	(very unquiet)
	20 Dec.	4 h 40' 1 mgm. Sc. + 3 cgm. Mo.........	5 h 20' to 7 h 40'
Br.	5 Dec.	4 h 2 mgm. Sc. + 2 cgm. Mo.............	6 h to 6 h 45'
	9 Dec.	4 mgm. Sc...............................	—
	12 Dec.	4 mgm. Sc...............................	—
	14 Dec.	4 cgm. Mo...............................	—
	16 Dec.	6 cgm. Mo...............................	—
	20 Dec.	4 h 50' 2 mgm. Sc. + 3 cgm. Mo.........	5 h 45' to 8 h 15'
Ba.	6 Nov.	2 h 30' 1 mgm. Sc. + 2 cgm. Mo.........	2 h 45' to 7 h 40'
	12 Dec.	2 mgm. Sc...............................	—
	16 Dec.	4 cgm. Mo...............................	-
	20 Dec.	3 h 40' 1 mgm. Sc. + 2 cgm. Mo.........	5 h 30' to 7 h 45'
Be.	12 Dec.	3 mgm. Sc...............................	—
	14 Dec.	4 cgm. Mo...............................	—
	16 Dec.	6 cgm. Mo...............................	—
	20 Dec.	4 h 30' 1½ mgm. Sc. + 3 cgm. Mo........	5 h 25' to 6 h 40'
E.	5 Nov.	4 h 1 mgm. Sc. + 2 cgm. Mo.............	5 h to 7 h 45'
	12 Dec.	2 mgm. Sc...............................	—
	16 Dec.	4 cgm. Mo...............................	—
	20 Dec.	4 h 40' 1 mgm. Sc. + 2 cgm. Mo.........	5 h 25' to 8 h 30'
F.	9 Dec.	3 mgm. Sc...............................	—
	14 Dec.	4 cgm. Mo...............................	—
	20 Dec.	4 h 45' 1½ mgm. Sc. + 2 cgm. Mo........	5 h 20' to 8 h 15'
He.	4 Nov.	4 h 1½ mgm. Sc. + 2 cgm. Mo............	5 h 15' to 8 h 45'
	12 Dec.	3 mgm. Sc...............................	—
	18 Dec.	5 cgm. Mo...............................	—
	20 Dec.	4 h 50' 2 mgm. Sc. + 2 cgm. Mo.........	5 h 25' to 9 h 25'

Hauckold worked with rabbits. The drugs were injected subcutaneously and then the behavior of the animals was observed for some time. Hauckold found, that whereas large doses

of scopolamine or morphine alone do not lead to a narcotic effect, 0.5 mgm. of scopolamine would intensify the action of 5 or 10 mgm. of morphine so that narcosis resulted.

One of the present writers in collaboration with Miss v. d. Made (4) showed that when the matter was investigated with accurate methods (studying the influence of the drugs on the reflexes of an isolated rectus femoris preparation in rabbits (5)) no evidence of any synergic effect was obtained. Moreover when they used Hauckold's method and also the same doses which in Hauckold's experiments had produced synergism, not a trace of "Potenzierung" was found.

In 1903 Kochmann (6) published an experiment on a dog of 6 to 7 kgm. where 10 mgm. morphine plus 0.5 mgm. of scopolamine gave "ein tiefer mehrstündiger Schlaf," whereas 10 mgm. morphine alone only produced "eine gewisse Benommenheit" and 0.5 mgm. scopolamine alone only gave a short superficial sleep without analgesia.

In the communication mentioned above (4) Storm van Leeuwen and Miss v. d. Made proved also that in the dog there is no potentiated synergism of scopolamine-morphine, in fact the result of the injection of combinations of these drugs is usually an algebraic addition of the effect of the two drugs.

As far as could be shown then in laboratory animals there was no potentiated synergism with scopolamine-morphine combinations and so there remained a discrepancy between these laboratory findings and those of Schneiderlin in men mentioned above.

We deemed it desirable therefore to investigate the matter again in monkeys as we hoped that on the one hand the results obtained in these animals would be in a closer connection to conditions in the human body, whereas on the other hand experiments on monkeys can be made more exactly than is possible in men.

We experimented on 5 monkeys (Macacus cynomolgus), obtained through the kindness of Dr. Noordhoek Hecht, director of the institute Pasteur, Weltevreden, Java. The animals were in good health and weighed 2 to 3 kgm.

In preliminary experiments on the action of morphine and scopolamine alone we found that doses of 10 mgm. morphine

produced symptoms of drowsiness though in no instance a real sleep ensued.   Usually when one comes near the cage of the animals and especially when one introduces an iron rod into the cage, the monkeys become furious, will bite the rod and so forth. The animals who had obtained 10 mgm. of morphine however were much quieter, they allowed themselves to be touched, they yawned frequently, but as stated above, they never really slept.

On the basis of these experiments it can be stated that the sensitiveness of Macacus cynomolgus to morphine does not differ materially from that in men.   The monkeys having one-twentieth of the weight of a normal adult showed definite symptoms after 10 mgm. of morphine.   In men doses of 60 mgm. (vide table 1) and more are often tolerated without producing many external symptoms.   With scopolamine however the matter is absolutely different.   We found that doses from 50 to 200 mgm. administered subcutaneously to our monkeys weighing 2 to 3 kgm. did not produce any perceptible effect.   One monkey which received a dose of 500 mgm. subcutaneously died within half an hour, showing loss of consciousness and marked spasms of the muscles of the extremities and of the trunk, death being caused very probably by spasm of respiratory muscles.   This very marked insensitiveness of the monkey for scopolamine was rather astonishing to us.   Generally it is thought that the sensitivneess to drugs acting on the central nervous system is to a certain degree dependent on the development of this system, higher animals being more susceptible than lower ones.   Thus the rabbit is very insensitive to scopolamine, the dog is much more sensitive, whereas in men doses of 4 mgm. and less have sometimes caused death.

It was to be expected that the monkey would in this respect take a position intermediate between the dog and man.   As it is this animal is highly insensitive to the drug.   An explanation of this fact cannot be given.   This matter is under investigation in this institute at this moment and the results of the research will be published later.

Although the monkey is very insensitive to scopolamine we were anxious to determine whether this drug could augment the action of morphine.   We therefore gave to 2 monkeys 5 mgm. of

morphine plus 5 mgm. scopolamine and to 3 other monkeys 5 mgm. morphine plus 50 mgm. scopolamine. All these injections as well as those mentioned above were given subcutaneously near the root of the tail. We took care always to leave an interval of several days between subsequent injections in the same animal. To rule out the possibility that by a former injection the animal had become accustomed to morphine, we gave a dose of morphine to all the animals some days after the end of the investigation, when they all reacted in the same way as they had done before. As can be seen from table 2 there was not even a trace of a "po-

TABLE 2

DRUG	MONKEY NUMBER				
	I	II	III	IV	V
5 mgm. morphine HCl..................................	−	−	+	−	−
10 mgm. morphine HCl.................................		+	++	+	+
30 mgm. scopolamine..................................		−			
40 mgm. scopolamine..................................			−		
50 mgm. scopolamine..................................					−
100 mgm. scopolamine.................................				−	
200 mgm. scopolamine.................................	−				
5 mgm. morphine plus 5 mgm scopolamine				−	−
5 mgm. morphine plus 50 mgm. scopolamine...........	−	−	+		

− No visible action.
+ Animal distinctly quieter, reacts less strongly to stimuli than normal animals.
++ Only slight reaction to stimuli, animal yawns and is sleepy.

tentiating" effect of scopolamine on morphine action in Macacus cynomolgus.

Table 2 shows distinctly that the action of 5 mgm. of morphine plus 5 mgm. of scopolamine and the action of 5 mgm. of morphine plus 50 mgm. of scopolamine is exactly the same as the reaction of 5 mgm. morphine alone and is much less than the action of 10 mgm. morphine, so that scopolamine does not intensify the action of morphine on the monkey.

After having found that scopolamine had so very little action on the monkey the question rose, whether the brand used by us was of good quality. To test this point we injected in a dog 1

mgm. of scopolamine per kilogram of animal subcutaneously; this had a distinct action of the same intensity as noticed in former experiments with other samples of scopolamine, so that we were sure that the scopolamine used was of good quality.

CONCLUSION

Monkeys (Macacus cynomolgus) are sensitive to doses of 5 to 10 mgm. morphine, given subcutaneously; they are very insensitive to scopolamine, doses of 200 mgm. of this drug given subcutaneously not producing any visible effect. A dose of 500 mgm. given to one animal proved to be fatal.

Scopolamine does not augment the action of morphine on the monkeys as far as concerns the external symptoms produced by this drug.

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THE RELATION OF HISTAMINE TO INTESTINAL INTOXICATION

I. THE PRESENCE OF HISTAMINE IN THE HUMAN INTESTINE

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INTRODUCTION

During the past two decades much has been written concerning intestinal intoxication. This appelation has been given to many diverse clinical conditions and there has seemed to be much confusion as to the exact scope of its meaning. It may be stated briefly that it must be due to either a bacterial or a chemical cause. The intoxication might result from either the direct action of bacteria or their endogenous or exogenous toxins per se, or else from toxic substances resulting from the action of the bacteria on the contents of the bowel. If these toxic substances are formed by such action on the contents of the bowel they are most probably derived from the end products of normal digestion. These substances may be presumed therefore to be such as would result from the perversion or undue prolongation of the action of the bacteria whose rôle may be normally to aid complete digestion. Although so-called intestinal intoxication resulting from bacteria and their direct products has been cited frequently as the cause of many indefinite diseased conditions it has not been possible as yet definitely to identify the offending organisms or to define their toxic properties, except in such well defined diseases as typhoid fever, dysentery, cholera, botulism, etc. On the other hand, there is accumulating rapidly both clinical and experimental evidence to prove that food-stuffs and their normal

455

derivatives may be toxic for one individual while not for another. Such facts are indicative of an anaphylactic condition in the individual, who is thereby inordinately susceptible to certain substances to which the ordinary individual is perfectly indifferent from a toxic point of view. This condition cannot in the broadest sense be called an intoxication. It is in general more a pathological susceptibility to an ordinarily innocuous substance than a general intoxication. Furthermore, such a condition of susceptibility may be manifest only if the substance to which the individual is sensitive be introduced through a certain avenue.

Under varying conditions the intestinal bacteria may exert different actions. If the habitat be anaerobic these bacteria have a more or less common property of splitting off the amino group from the protein molecule, but if the surroundings be aerobic the carboxyl group is first separated. It is of great practical importance whether this separation of the carboxyl group takes place after or before deamidization. If after, substances such as indol and phenol are formed which have little or no toxic properties. On the other hand, if the separation of the carboxyl group takes place before the amino group is split off very toxic substances such as histamine, tyramine, etc., are formed. At present there is no direct proof that such substances are formed in the human gut, or if they are that they are absorbed by the intestinal tract; but it is conclusively proven that they exert a violently poisonous action if inoculated subcutaneously or intravenously into animals.

As has been already stated, bacterial putrefaction possibly plays a certain rôle in complete digestion. This takes place practically entirely in the cecum and the proximal part of the transverse colon. In considering the various end products of the putrefactive digestion which might under abnormal circumstances exert a toxic action on the organism in general, we selected those resulting from the digestion of protein and in particular the amines which might result from bacterial decarboxylation of the amino acids. It was decided therefore to seek for the presence of histamine, not that it was considered a priori that this was necessarily a causative agent of intestinal toxemia under normal

conditions, but since if it were found that this and other possibly toxic substances were present normally in the cecal contents, under abnormal conditions of structure and function, these might be proved operative as toxic agents.

Histamine has been isolated by Barger and Dale (1) from the intestinal mucosa of the bullock; moreover Mutch (2) has obtained from the human ileum an organism (unidentified) which is capable, under aerobic conditions, of decarboxylating histidine. In consequence of these observations the view has been held by many that, in certain cases, histamine might be the toxic agent, especially in view of the fact that one of the symptoms frequently observed was a chronic low blood pressure, such as conceivably might be produced by the continuous absorption of small amounts of histamine. The present investigation was undertaken in the hope of obtaining clearer evidence on this point by the isolation of histamine from the contents of the human intestine, or, failing this, by obtaining definite proof of its presence or absence therein.

This proof we proposed to obtain by means of the very sensitive reaction to histamine which is given by the isolated uterus of the virgin guinea-pig. Although this reaction in itself is not a specific test for histamine, we consider that in this case it may be regarded as such, in view of the preliminary chemical treatment to which we subjected our material; so that, although in no case were we able to obtain enough material for the chemical isolation of histamine, we consider that, in those cases in which we obtained a positive physiological reaction, its presence is established.

EXPERIMENTAL PART

The material investigated consisted of cecal contents (cases 1, 2, 3, 6), contents of the transverse colon (cases 4 and 5), and feces (cases 7 and 8) of patients in the Royal Infirmary, Edinburgh. The cecal and colonic contents were obtained by washing out the cecum (or colon) or were collected on dressings. On collection they were treated as follows: In the case of caecal washings these were treated at once with mercuric chloride and

hydrochloric acid to make a concentration in the whole of 0.5 per cent of the former and 0.9 per cent of the latter; when the material was obtained on dressings, the latter were placed at once on removal in a solution of mercuric chloride and hydrochloric acid of the above mentioned concentrations; in dealing with feces (formed stools) these were thoroughly broken up and mixed with water, mercuric chloride and hydrochloric acid being added as before. In this manner it was possible to accumulate quantities of material which were fixed as regards bacterial reaction, at the stage which they had already reached in the intestine.

When a considerable quantity (1500 to 2500 cc.) of material had been collected in this way, the whole mixture was boiled and filtered; this filtration was sometimes troublesome, and was almost impracticable without previous boiling.

For the further working up of the material the first method tried was to make the solution aklaline in order that any bases present might be precipitated by the excess of mercuric chloride; this however was not found to be satisfactory.

As a result of numerous experiments the following was found to be the most satisfactory procedure. After the first filtration the solution, which was clear, but in most cases highly colored, was freed from excess of mercury by means of hydrogen sulphide; the mercuric sulphide was filtered off and the filtrate treated with lead acetate (first a solution of neutral lead acetate and then of the basic salt). This produced a bulky precipitate which was filtered off and well washed with water. The solution was then freed from excess of lead by means of hydrogen sulphide and the lead sulphide was filtered off.

At this stage the solution was almost colorless or light yellow. It was now concentrated on the water bath in vacuo, the temperature being kept below 60°C., to a small volume; sulphuric acid was added to make a concentration of 5 per cent and the solution was treated with phosphotungstic acid until there was no further immediate precipitate; after standing over night the precipitate was separated by the centrifuge, washed, dissolved in a mixture of acetone and water, and decomposed with baryta.

The filtrate from the barium phosphotungstate was completely freed from barium with sulphuric acid, and was concentrated in vacuo, most of the acetone being removed by this means. The solution thus obtained was now fractionated with silver nitrate and baryta.

The prcipitate obtained in the histidine fraction was separated by the centrifuge, well washed with water, suspended in distilled water, and decomposed with hydrogen sulphide; the silver sulphide was filtered off and washed, and the united filtrate and washings were thoroughly freed from excess of hydrogen sulphide by boiling under reduced pressure.

It was subsequently found that the precipitation with phosphotungstic acid might, with advantage, be omitted.

In cases 1 to 6 the solution, obtained on recovery from the silver precipitate, gave Pauly's reaction with sodium diazobenzene sulphonate, but with considerable variations in intensity. In cases 7 and 8 no diazo reaction could be obtained.

The solutions at this stage were tested for physiological activity as follows: A horn of the uterus of a virgin guinea-pig was suspended in a bath of oxygenated Ringer-Locke solution at 37°C. The sensitiveness of the uterus to histamine was first determined by finding the amount of the latter which it was necessary to add to the bath in order just to produce a contraction; the process was then repeated, substituting the solution to be tested for the histamine. In this way it was possible to form an approximate idea of the concentration of histamine present.

In order to eliminate the possibility of confusion between histamine and the other substances of unknown constitution, which give a similar reaction with the uterus, part of the solution in two typical cases (1 and 6) was heated in the boiing water bath for ten minutes with a concentration of 4 per cent sodium hydroxide; the physiological test was then repeated on this solution after cooling and neutralization. In case 1 a certain diminution of activity was observed but in case 6 no such diminution could be found. There was therefore present some physiologically active substance, which, having regard to its

method of isolation, and also to the fact of its resistance to hydrolysis, must be presumed to be histamine. The variations in physiological activity in the different cases corresponded closely with the variations in the intensity with which the diazo-reaction was given.
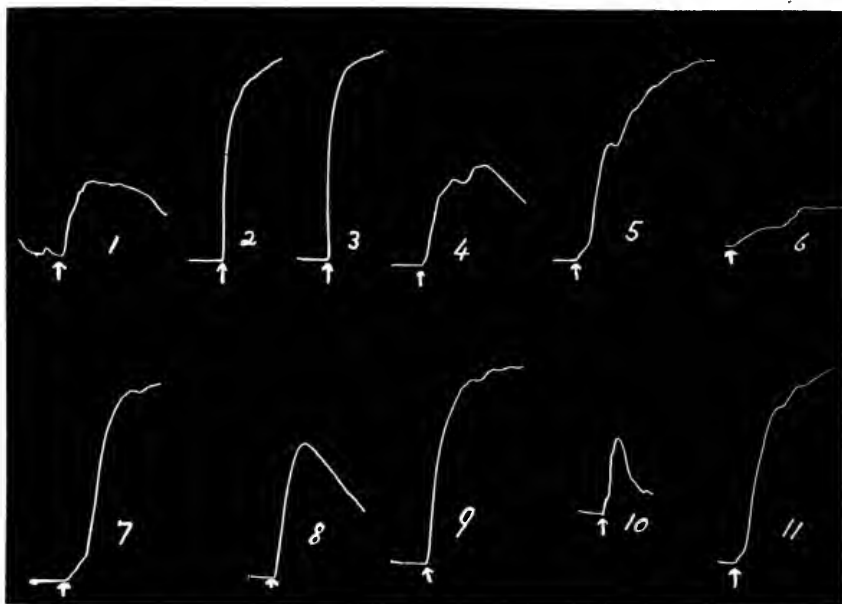
FIG. 1

1. Histamine solution, dilution,* 1 : 16.6 millions = submaximal contraction.
2. Histamine solution, dilution 1 : 12.5 millions = maximal contraction.
3. Histamine (hydrolysed) solution, dilution 1 : 12.5 millions = maximal contraction.
4. Case 1, non-hydrolysed fraction. Dilution 1 : 85 = submaximal contraction.
5. Case 1, non-hydrolysed fraction. Dilution 1 : 72 = maximal contraction.
6. Case 1, hydrolysed fraction. Dilution 1 : 50 = submaximal contraction.
7. Case 1, hydrolysed fraction. Dilution 1 : 37 = maximal contraction.
8. Case 6, non-hydrolysed fraction. Dilution 1 : 335 = submaximal contraction.
9. Case 6, non-hydrolysed fraction. Dilution 1 : 250 = maximal contraction.
10. Case 6, hydrolysed fraction. Dilution 1 : 278 = submaximal contraction.
11. Case 6, hydrolysed fraction. Dilution 1 : 250 = maximal contraction.

* All dilutions indicate the concentrations in the bath of solutions or fractions.

Case 1. M. M. Female. Age forty. This patient had suffered for many months with irregular fever and intermittent distention of the abdomen. An exploratory operation on July 29, 1920, was performed and nothing abnormal was found. She was admitted to the Royal Infirmary on September 16, 1920, and while under observation symptoms of acute intestinal obstruction developed. Mr. Graham operated on September 22, 1920, and although the whole of the cecum and colon were found greatly distended, no definite point of obstruction was evident. A cecostomy opening was made which continued to function satisfactorily and between December 24 and 29, 1920, the material discharged from the cecostomy was collected and examined for histamine. On further examination there was found evidence of obstruction at the splenic flexure. A second operation was performed by Mr. Jardine on March 19, 1921, when adhesions and kinking of the splenic flexure were found, producing acute intermittent obstruction. Anastomosis was performed at this site and the patient made an uninterrupted recovery.

Case 2. Mrs. I. Age fifty-four. This patient gave a history of intermittent diarrhea without constipation covering a period of one year. On January 10, 1921, she developed signs of acute intestinal obstruction. At operation on January 15, 1921, Mr. Graham found great distention of the small intestines, cecum, transverse descending and upper part of pelvic colon. In the lower part of the pelvic colon a malignant stricture was found. Cecostomy was performed which continued to function satisfactorily for some months, when the patient returned to have the malignant growth removed. On February 5, 1921, the contents discharging from the cecostomy opening were collected and examined for the presence of histamine.

Case 3. A. T. Male. Age fifty-nine. For some years this patient had been troubled with dyspepsia but early in February, 1921, he became acutely ill with severe pain in the lower abdomen and vomiting, followed by obstinate constipation in spite of purgatives. Enemas however gave satisfactory results, relieving the vomiting. On March 7, 1921, an operation was performed by Sir Harold Stiles and there was found a malignant stricture at the junction of the iliac and pelvic colon, while the cecum, transverse colon and descending colon were much distended. Cecostomy was performed which continued to function satisfactorily, On April 1, 1921, and again on April 10, 1921, the contents from the cecostomy were collected and examined for histamine.

Case 4. G. L. K. Age sixty-seven. For several months the patient had complained of obstinate constipation with pain in the lower abdomen

which was paroxysmal in character and frequently accompanied by vomiting. On February 20, 1921, symptoms of complete obstruction developed and he was admitted to the Royal Infirmary on March 1, 1921, for intestinal obstruction. At operation Sir James Hodsdon found a malignant growth with many adhesions involving the gall-bladder, liver and transverse colon. A colostomy opening was made about the middle of the transverse colon which continued to function satisfactorily. On April 4, 1921, material from colostomy was collected and examined for histamine.

Case 5. Mrs. J. G. Age sixty-two. For several months patient had complained of epigastric pain vomiting and constipation. On February 15, 1921, these symptoms became very extreme and signs of acute intestinal obstruction developed. Patient was admitted on this date and Sir James Hodsdon operated immediately, when a carcinoma of the upper part of the pelvic colon was found. A preliminary colostomy opening was made in the transverse colon which continued to function satisfactorily. On April 1, 1921, material from the colostomy was collected and examined for histamine.

Case 6. T. E. Male. Age forty-eight. In September, 1919, patient began to suffer from pain across the lower abdomen with flatulence and gaseous eructations. He gradually became more and more constipated. Suddenly in October of that year the constipation became complete and he had great distention of the abdomen, followed by vomiting. He was admitted to the Royal Infirmary on October 13, 1919, when he was operated on by Mr. Wilkie for carcinoma of the pelvic colon. At operation this diagnosis was confirmed, a large tumor was removed and colostomy was performed. On December 23 patient returned to the Infirmary and was again operated upon by Mr. Wilkie and the colostomy closed. He continued in good health for about a year, when he was readmitted to the Infirmary on March 31, 1921, suffering from recurring attacks of pain in the abdomen, which was relieved by the passage of flatus. On examination the abdomen was distended, very tympanitic, with tenderness and rigidity in the right iliac fossa. A tumor was palpable which was hard, irregular and adherent to the scar of the previous operation. There were pronounced constipation and recurrent attacks of vomiting. On April 1, 1921, patient was operated on by Mr. Wilkie. On opening the abdomen a considerable quantity of yellowish fluid escaped and numerous nodules were found in the peritoneum and in the omentum, which were found to be carcinomatous, and there was a large mass found in the right

iliac fossa. The cecum was brought to the surface and cecostomy performed. This continued to function very well and on April 16, 1921, the contents from the cecostomy were collected and examined for histamine.

Case 7. K. McK. Male. Age thirty-two. For fifteen years this patient had been troubled with headaches and "biliousness" with increasing mental depression. In 1917 his appendix was removed and some months later he was operated upon for intestinal obstruction when 3 feet of the ileum was resected. After these operations he suffered from diarrhea. In the spring of 1920 he was again operated on for the separation of adhesions, after which he had temporary relief. In September, 1920, another operation was performed by Mr. Wilkie, when it was deemed advisable to do a short-circuiting and the terminal portion of the ileum was anastomosed to the upper part of the ascending colon. His condition did not improve and he continued to suffer from sleeplessness, headache and profound nervous depression. A barium meal demonstrated the cecum to be large and flaccid and apparently in a very atonic condition. It would fill with ease up to the level of the ileo-colonic opening, when there would be distinct syphonage and regurgitation into the ileum. A barium enema demonstrated this regurgitation in a most pronounced degree. The stools were collected from January 15 to January 20, 1921, and examined for histamine.

Case 8. M. B. Female. Age ten. This patient suffered from chronic nephritis without any evidence of intestinal disturbance. She was on milk diet. On January 13 to January 15, 1921, her stools were collected and examined for histamine.

We were able to demonstrate the presence of histamine in cases 1 to 6 but not in cases 7 and 8; i.e., in the contents of the cecum and transverse colon but not in the feces. We obtained the largest amount in case 2, where the activity of the solution, by comparison with one of pure histamine, corresponded with a concentration of 1:10,000 of the latter.

The complete record of the physiological test in the typical cases 1 and 6 is shown in figure 1 and the other cases are recorded in table 1. Owing to the uncertainty of the amount of dilution of the original material, and the far from quantitative nature of the method of working up, it is impossible to form an accurate esitmate of the concentration in which histamine was

present in the intestine.   In case 1, from a consideration of
the various factors, we arrived at 1:100,000 as being a figure
expressing the order of magnitude of the original concentration.

TABLE 1

CASE NUM- BER	NATURE OF ORIGINAL MATERIAL	APPROXIMATE VOLUME OF SOLUTION IN HISTIDINE FRACTION	RESULTS OF TESTS	APPROXIMATE CONCENTRATION OF HISTAMINE IN FRACTIONS
		cc.	cc.	
1	Cecal washings	50	0.6 + 0.7 ++	1 : 350,000
2	Cecal washings	50	0.1 ++	1 : 10,000
3	Cecal washings and dressings.....	70	2.0 ++	1 : 1,000,000
4	Contents of transverse colon (on dressings)	50	0.05 ++	1 : 25,000
5	Same as 4	100	0.40 + 0.50 ++	1 : 250,000
6	Cecal washings	80	0.15 + 0.20 ++	1 : 50,000
7	Feces...........................	50	Entirely negative	0
8	Feces...........................	50	Entirely negative	0

Column 4 gives the volume of the solution to be tested, which was added
to the bath (capacity 50 cc.).
  + represents submaximal contraction of the uterus.
  ++ represents maximal contraction of the uterus.

We are at present engaged upon experiments dealing with the
absorption of histamine and its fate in the organism, the results
of which we hope to publish shortly.

SUMMARY

1. The presence of histamine, in minute concentration, is demonstrated in the cecum (four cases) and in the transverse colon (two cases).

2. The formation of histamine is apparently not dependent upon the existence of intestinal obstruction, since it occurs several weeks after the obstruction has been removed.

3. Histamine could not be detected in the feces, whether there was intestinal disturbance or not; we regard this as probably due to the oxidation of this substance during the passage through the large intestine.

REFERENCES

(1) BARGER AND DALE: Jour. Physiol., 1911, xli, 499.
(2) MUTCH: Quart. Jour. Med., 1914, vii, 442.

ERRATA

Bonar and Sollmann, Vol. XVIII, *page 467:*

The substance III, the chemical name of which was stated to us as "diethyl amino propyl diphenyl amino carbinol," possesses the formula

$$\begin{matrix} C_6H_5 \\ \diagdown \\ \diagup \\ C_6H_5 \end{matrix} N - COO - (CH_2)_3 \; N(C_2H_5)_2 \; HCl$$
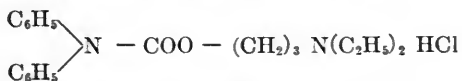
and should have been named "diphenyl carbaminyl gamma diethyl amino propanol."

On *page 469*, fifth line, read "beta" instead of "gamma."

THE EFFECTS OF SOME NEW LOCAL ANESTHETICS[1]

(PARA-AMINOBENZOYL DINORMAL BUTYL-AMINO ETHANOL AND PROPANOL; AND DIETHYL AMINO-PROPYL DIPHENYL AMINO-CARBINOL)

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INTRODUCTION

Of the large number of synthetic local anesthetics that have been introduced, none has quite attained the clinical ideals of universal efficiency and safety.

It is of course essential that the substance should be effective in practical concentrations. This can be easily determined by experiment; and no substance deserves further investigation until its anesthetic efficiency is established. A large number and considerable variety of substances paralyze sensory nerves on direct contact. None that has so far been introduced is as efficient as cocain from the surface of mucous membranes. None has been found satisfactory from the intact skin.

Anesthetic efficiency alone is not a sufficient criterion of the availability of a substance as a practical local anesthetic. The real criterion or "therapeutic index" would be the ratio of efficiency to toxic and other side actions. This, again, cannot be expressed as a single number; for efficiency and side actions both depend on the mode and site of application.

The current methods of estimating efficiency, toxicity and irritation are fairly simple in principle and execution. They supply data of fundamental scientific importance; and they may render considerable practical service by cutting short the career of distinctly unpromising anesthetics before they are introduced into clinical practice.

The final selection of the more valuable from the crowd of more or less promising local anesthetics is much more difficult. Definite criteria have not been established, and in their absence, it is necessary to feel one's way. Judgment must be cumulative, since it cannot, for the present, be absolute.

This investigation represents such an attempt to apply a number of experimental criteria to some new compounds that appeared at the time sufficiently promising to justify the attempt. These had been submitted to the Council on Pharmacy and Chemistry of the American Medical Association, by the Abbott Laboratories, for judgment, prior to their introduction into practice. The results showed that two of the substances are unpromising, whilst the third may have a limited field.

The investigation was more extensive, in some lines, than the importance of these particular substances would warrant; but it still falls short of what would be desirable if the substances had turned out more promising. We consider this point of view of more importance than the particular results obtained with these particular chemicals to which it was applied.

The actual experiments were performed by Bonar, according to the methods that had for the most part been used by Sollmann in the study of other local anesthetics. It will therefore not be necessary to describe the methods in detail; and the results may be presented in the form of the critical data and relations, usually without reproducing all the experiments that led up to these. In a few cases, however, the details are sufficiently important to be presented more extensively. This is done in connection with the description of the methods.

SUBSTANCES EXAMINED

These have been described by Kamm, Adams and Volwiler, 1920 (1), and Volwiler, 1921 (2). They were known to us only under code numbers, which we shall also use in the following descriptions, for the sake of brevity: "G" for the para-amino-benzoyl gamma dinormal-butyl-amino ethanol hydrochlorid. "H" for the corresponding propanol hydrochlorid, which was also used in the form of the more soluble succinate. (The soluble sulphate of this base has since been submitted to the Council under the name of "Butyn.") "III" for the diethyl amino-propyl diphenyl amino-carbinol hydrochlorid.

The amino-benzoylesters "G" and "H" are related to procain (novocain), para amino-benzoyl diethyl-amino ethanol hydro-chlorid; the "G" differing only by the replacement of the two ethyl by two butyl radicals; the "H" by the further substitu-tion of propanol for ethanol.

Solubility in water. This was stated by Volwiler as follows: "G," 1 per cent at 20°C.; "H," hydrochlorid: 0.5 per cent at 20°C, 1 per cent at 60°, which remains in solution on cooling; "H," succinate, 4 per cent at cold, "III," 10 per cent at 20°C.

Our data differ but slightly from these, namely: "III," procain, and cocain freely soluble in all percentages required. "G" dissolved up to 3 per cent when heated to 80°C., and not cooled below 20°C. Further cooling threw out of solution. "H" dissolved up to 1 per cent under same conditions as "G." Cooling threw out of solution. "H"-succinate dissolved to 4 per cent, above 20°C.

The solubility of the amino-benzoyl esters is therefore much lower than that of procain (1:0.7). This need not be a dis-advantage, provided that the efficiency is sufficiently high; it may even be an advantage by preventing such errors in dis-pensing too concentrated solutions, as have caused deaths in the case of cocain.

EXPERIMENTAL METHODS

For the greater part, the methods have been previously described. These may be summed up very briefly:

Anesthetic efficiency on motor nerves. Technic of Sollmann (3). Muscle nerve preparations were made from the frog's leg so as to include the lower end of the leg from the knee down, and the entire sciatic nerve from the knee to the spinal cord, with a bit of bone attached.

The preparations were laid in $\frac{M}{8}$ NaCl (0.73 per cent) made with tap water. In making the tests the entire nerve was immersed in the anesthetic solution, contained in a little trough cut in a block of paraffine. Each trough held about 1 cc. solution. The excitability was tested with the platinum electrodes of a Harvard induction coil, activated by a current of about 4 volts with the secondary at 12 cm.

The stimuli were applied at the distal end of the nerve, within 1 cm. of the spinal origin, at five minute intervals. The temperature was maintained between 17° and 20°C. All the frogs used came from the same lot.

The anesthetics were dissolved in $\frac{M}{8}$ (0.73 per cent) NaCl and the concentrations were in geometric ratio ($\frac{1}{4}$-$\frac{1}{2}$-$\frac{1}{2}$).

The nerve muscle preparations were tested every five minutes with the object to determine concentrations that paralyze in twenty minutes.

Anesthetic efficiency on sensory nerves. Technic of Sollmann (4). Frogs were decapitated, leaving the lower jaw. A transverse section was made through the abdomen just below the sternum, comprising about two-thirds of the circumference. The viscera were removed through this opening. The frogs were suspended by the lower jaws, and the eviscerated cavities, which formed little pockets were filled with small pledgets of cotton saturated with the anesthetic solutions. Further quantities of solutions were poured in to insure thorough saturation. About 5 cc. of each solution was applied in this way. The drugs were dissolved in $\frac{M}{8}$ NaCl solution, as in the preceding.

At five minute intervals the persistence of the reflex paths were tested by immersing the feet in $\frac{N}{10}$ HCl. Immersion was continued for one minute unless a reflex was sooner elicited. The legs were rinsed with tap water after each application.

Anesthetic efficiency of frog skin. Technic of Sollmann (5). The frogs were decapitated, leaving the lower jaw; heart excised; frogs suspended by the jaw. Each leg was immersed in a "homeopathic vial" containing about 6 cc. of anesthetic solution, reaching about midway to the knee. A rubber band placed around the legs below the hips prevented the frog from removing the legs from the solution.

At the end of every ten minutes the legs were withdrawn and the feet immersed in $\frac{N}{10}$ HCl. If the frog reacted sharply by jerking the feet from the acid, the legs were washed in water and replaced in the solution. This procedure was repeated at ten minute intervals during thirty minutes, or until paralysis was complete.

Anesthetic efficiency on rabbit cornea. Technic of Sollmann (6). Rabbits were confined in snugly fitting comfortable individual stalls, leaving the head free. The eye-lashes were clipped; winking reflexes confirmed by touching the cornea with the sharpened point of a soft lead pencil. The lower eyelid was pinched into a pocket, into which was inserted the point of an eyedropper filled with the anesthetic solution. The conjunctival sack was thus kept flooded for one minute. The dropper was then removed and the animal released. In ten minutes the winking reflex was tested by touching the cornea, near the center, with the pencil point. If this did not produce winking, the pencil was drawn across the cornea several times with moderate pressure, care being taken not to touch the lid or the surrounding hairs. Absence of winking was taken as the sign of complete anesthesia.

If at the end of the ten minute period the anesthesia was absent or only partial the conjunctival sack was again filled with the solution; but the rabbit was not held for a minute, being released at once.

Tests and applications were repeated every ten minutes until four applications had been made, unless complete anesthesia had been attained before. No applications were made after complete anesthesia. The tests, however, were continued until complete return of normal sensitiveness.

The solutions were made with $\frac{M}{8}$ (0.73 per cent) NaCl, as usual. Phenacain (holocain) and beta eucain, being extensively used in eye-work, were added to the list for comparison.

The data are shown in table 1. Under "A" is indicated the number of applications (at ten minute intervals) required to produce complete anesthesia; under "R" is indicated the num-

TABLE 1

Anesthesia of rabbit's cornea

DRUG	10 PER CENT		4 PER CENT		2 PER CENT		1 PER CENT		½ PER CENT		¼ PER CENT		⅛ PER CENT		MINIMUM EFFECTIVE CONCENTRATION	EFFECTIVE RATIO
	A	R	A	R	A	R	A	R	A	R	A	R	A	R	*per cent*	
Cocain................							1	30	2	30	2*	20	*		½	1
Procain................	1	35	*												10	$\frac{1}{10}$
Holocain..............							1	50	2	40	1	20	*		¼	2
Beta eucain...........					2	40	*		*						2	¼
"G"..................							1	30	*		*				1	½
"III".................					1	35	1*	30	*		*				2	¼
"H"..................									1	30	1*	40	*		½	1
"H" succinate.........							1	30	1	20					½	1

* Incomplete

ber of minutes elapsing between the last application and complete recovery of sensation. "Incomplete" signifies that the cornea is evidently less sensitive, but that a reaction occurred.

In table 1 the percentage determining the minimum effective concentration is that which causes complete anesthesia with one or more applications. The efficiency ratio is varied in a moderate degree depending on the concentration and the number of applications.

In the above table 1 the ten minute interval may have been perhaps too long between the application of anesthetic and the testing for sensation. Some recovery may have meanwhile occurred. Table 2 suggests this.

Anesthetic efficiency and irritation—human cornea. One drop of the anesthetic solution was applied to the eye. Tests for sensitivity were made every minute, by drawing the tip of a

TABLE 2

Human cornea

DRUG	ANES-THESIA DURATION	RETURN OF SENSI-TIVITY	EFFI-CIENCY	OBSERVATIONS
	minutes	*minutes*		
Cocain, 1 per cent.......	*	5		
Cocain, 2 per cent.......	6	11	1	Slight burning with lachrymation for 30 seconds. Sclera blanched. Dilatation of pupil began in 5 minutes
Procain, 1 per cent......	0			
Procain, 2 per cent......	0		0	Nothing characteristic
Holocain, 1 per cent.....	5	9	1+	Sharp burning for 30 seconds. Conjunctiva moderately injected. Irritation persisted $\frac{1}{2}$ hour
Beta eucain, 1 per cent..	*	4	?	Burning for $1\frac{1}{2}$ minutes. After return of normal sensation felt dry, and burned
"G," 1 per cent.........	4	8	1+	Severe conjunctival burning for 2 minutes. Eye later red and felt irritated
"III," 1 per cent.......	*	5	?	Very marked burning, much lachrymation, persistent
"III," 2 per cent.......	*	4	?	conjunctival irritation and injection
"H," $\frac{1}{4}$ per cent........	2	8	2+(?)	Warm soothing feeling. No subsequent irritation. Sclera not blanched. No dilatation of pupil

* Incomplete.

tooth-pick well covered with a tightly twisted whisp of absorbent cotton, across the cornea; care being taken not to touch the lashes. If touching, or gentle rubbing caused no subjective sensation, anesthesia was considered complete. Observations,

both subjective and objective, were made upon two different individuals with almost identical results. The observations were made at not less than two hours apart on a given eye. The data are shown in table 2.

Anesthetic efficiency for human, intracutaneous injection. Technic of Sollmann (7). The flexor surface of arm was scrubbed

TABLE 3

Intracutaneous anesthesia, human

DRUG	DURATION COMPLETE ANESTHESIA			EFFECTIVE RATIO	OBSERVATIONS
	Ulnar wheal	Mid wheal	Radial wheal		
	minutes	*minutes*	*minutes*		
Cocain, $\frac{1}{32}$ per cent.	8	5	4	1	Blanched areola with pink center; irregular blanched streaks radiating from areola. No irritation on injection
Cocain, $\frac{1}{64}$ per cent.	0	0	0		
"G," $\frac{1}{32}$ per cent...	5	2	0	1—	Very slight irritation. Moderate blanching, no streaking
"H," $\frac{1}{32}$ per cent...	10	6	5		
"H," $\frac{1}{64}$ per cent...	4	11	2	2—	Complete blanching, turned uniformly pink in 2½ hours. No pain on injection or subsequently
"H," $\frac{1}{128}$ per cent..	0	0	0		

Observations were not made on the sample of "III" because of the irritation caused by a preliminary injection.

with alcohol. Injections were made into the skin with a fine needle attached to a Luer syringe. Three wheals were made across the arm, 7 to 10 mm. in diameter, each requiring about 0.3 cc. of solution. These wheals were tested with a bit of cotton twisted to a fine point. Solutions were made up with epinephrin in 1:100,000 dilution, in 0.73 per cent NaCl.

The data are shown in table 3.

Minimum fatal dose, intravenous cat method. This was deter-
mined for "H" and "III" by Professor Hatcher of Cornell
Medical School, according to the method used by him and
Eggleston (8) for other local anesthetics, i. e., rapid injection
of 1 to 2 per cent solution into the femoral vein, under local
anesthesia by phenol.

As regards "H" Hatcher concludes:

The experiments with Anesthetic "H" seem to show that the fatal
dose for the cat by rapid intravenous injection of a 1 per cent solution
is approximately 15 mgm. per kilo of weight, but that much smaller
doses cause marked respiratory disturbance and that there is a marked
convulsive action with doses as small as 5 mgm. per kilo.

Doses of 10 to 12.5 mgm. per kilo were so nearly fatal that the two
fatal injections seem to fix the fatal dose sufficiently for the present
purpose unless for any reason you wish it determined more accurately.
The drug is not eliminated, nearly as promptly as procain (at least,
recovery is not so rapid), but elimination appears to be practically
complete in an hour and half.

The details are illustrated by the protocols from two of the
seven animals.

November 13. Experiment 5. Male, 1.26 kgm.
4.24 p.m. 15 mgm. per kilogram injected intravenously in sixteen
  seconds. Struggling began during injection.
4.25 p.m. Heart slow, soon becoming irregular, gradual improve-
  ment; single convulsive jerks of the whole musculature.
4.28 p.m. Severe clonic convulsions lasting about twelve minutes,
  after which occasional twitching of limbs.
5.00 p.m. Released; sits up; purrs.
November 14. Next day; 1.21 kgm. (loss 0.05 kgm.)
9.51 a.m. 15 mgm. per kilogram injected in six seconds; cry of
  distress at once.
9.54 a.m. Respiration slow, feeble; convulsive jerks of body with
  inspiration, becoming more frequent, accentuated about
  head.
10.10 a.m. Convulsions have ceased (exact time of stopping not
  recorded).
11.21 a.m. 15 mgm. per kilogram as previously (one hour and thirty
  minutes after previous dose); after about one minute

there began a cycle of respiratory changes that continued for nearly an hour, the movements becoming progressively feebler; the respiration would be nearly normal, rapidly increasing in rate until about 200 to 300 per minute, then suddenly ceasing and beginning with convulsive gasps, these in turn lessening till the animal appeared to have expired, only to assume a nearly normal respiration and going through the cycle in about two minutes or less. The heart was relatively strong, the pulse being palpable in the femoral region until shortly before death.

11.52 a.m.   Respiration only in gasps, cycle becoming less distinct.

12.17 p.m.   Heart stopped; no respiration, or only the feeblest observed for several minutes. Death due mainly, or exclusively, to respiratory failure

Experiment 6. Female, 2.26 kgm.

9.37 a.m.   10 mgm. per kilogram as in previous experiments. Violent struggling interrupt injection twenty seconds after starting; intended to inject larger dose; convulsions lasting about five minutes.

9.55 a.m.   Respiration rapid at this time. This animal, like all that received large doses, frothed at mouth during convulsions. Animal became normal and was subsequently used for another drug.

Regarding compound "III," Hatcher reports:

The fatal dose lies between 10 and 15 mgm. per kilogram; marked tendency to acquire rapid tolerance was observed with various other local anesthetics by Eggleston and Hatcher, and thus drug appears to have the same property of inducing slight tolerance after one or two doses; data not sufficient for final decision.

Fatal dose of compound for cat by subcutaneous injection in concentrated solution (50 mgm. per centimeter) lies not far from 50 mgm. per kilogram, apparently.

Recovery follows small intravenous doses in a few minutes, but the recovery is not complete since violent convulsions and lasting depression followed the injection of a total of 47.5 mgm. per kilogram in 6 doses in a period, of ninety-six minutes; the behavior in this respect is strongly suggestive of cocain (see paper of Eggleston and Hatcher).

Details are illustrated by the protocols of three of the experiments:

Experiment 3. Female, 2.61 kgm.

11.40 a.m.   Injected about 5.0 mgm. per kilogram intravenously but some lost; animal released.

1.24 p.m.   Injected 50 mgm. per kgm. (50 mgm. per cubic centimeter) subcutaneously (not intramuscularly) in abdominal region.

2.14 p.m.   Salivation and distress; slight twitching of ears.

3.05 p.m.   Violent clonic convulsions.

3.40 p.m.   Muscular incoördination, unable to walk without swaying; struck head (due to incoördination) and instantly had violent clonic and tetanic convulsions. Had shown symptoms previously.

Experiment 4. Female, 2.50 kgm.

11.57 a.m.   Injected 5 mgm. per kilogram. (20 mgm. per cubic centimeter) into femoral. Cried as if in distress, but not absolutely certain of cause. No other symptom (if this is to be regarded as a symptom of circulatory distress).

12.02 p.m.   Injected 5 mgm. per kilogram as before; greater apparent distress than from previous dose.

12.07 p.m.   Injected 5 mgm. per kilogram as previously; symptoms as before.

12.37 p.m.   Injected 7.5 mgm. per kilogram as previously; symptoms as before.

1.13 p.m.   Injected 10 mgm. per kilogram as previously; convulsive movements; heart and respiration not stopped.

1.33 p.m.   Injected 15 mgm. per kilogram as previously; clonic convulsions; frequent during about fifteen minutes; released.

1.49 p.m.   Violent clonic convulsions repeated during several minutes.

3.12 p.m.   Barely able to stand.

4.20 p.m.   Walks very feebly.

5.00 p.m.   Destroyed with chloroform.

Experiment 5. 1.56 kgm.

2.00 p.m.   Injected 10 mgm. per kilogram as in other experiments (20 mgm. per cubic centimeter); violent respiratory efforts and rapid recovery.

2.18 p.m.   Injected 15 mgm. per kilogram as previously; death in three minutes with symptoms as described above.

TABLE 4

Hypodermic injections; white rats

DRUG		MGM./KGM. WEIGHT	SEX	AGE	WEIGHT	RESULT	REMARKS	DATE
Cocain	a	100	M	Adult	225	Died	9 hours alive; excited; 24 hours dead	10-15
	b	200	M	Young	173	Recovered	Excited	10-16
	c	200	M	Young	167	Recovered	Excited	10-21
	d	400	F		140	Recovered	Excited; 24 hours dopey	10-23
	e	400	M	Young	138	Recovered	Excited	10-27
	f	600	M	Young	182	Died	6 hours alive; 24 hours dead; same rat as b	10-27
	g	600	F	Young	212	Died	24 hours alive; timid; 29 hours dead	10-29
Procain	a	100	M	Adult	271	Recovered	Curious; stiff	10-15
	b	200	M	Young	150	Recovered		10-16
	c	200	M	Adult	271	Recovered	Same rat as a	10-21
	d	400	F	Pregnant	147	Recovered		10-23
	e	600	M	Young	152	Recovered	Same rat as in a	10-29
	f	800	F	Pregnant	188	Recovered	Unable to walk in 1½ hours, 24 hours OK	10-29
	g	1000	M	Young	143	Recovered	2 to 6 hours unable to crawl; same rat as e	10-30
	h	1500	F		150	Died	½ hour unable to walk; 3 hours dead	10-31
	i	1250	F		153	Died	Convulsions in 3 minutes; 6 hours dead	11-1
III	a	100	M	Adult	195	Recovered	Rough coat; whined	10-15
	b	200	M	Young	156	Recovered	Leg stiff; hip caked	10-16
	c	200	M	Adult	190	Recovered	Same rat as a	10-21
	d	300	F	Pregnant	241	Recovered	No symptoms after 3 hours	10-24
	e	400	F	Pregnant	178	Died	Dead in 5 minutes	10-23
	f	400	M	Young	140	Recovered	15 minutes could scarcely walk	10-29
	g	400	F	Pregnant	153	Died	36 hours dead; alive over 24 hours	10-29

"G"	a	100	M	Adult	250	Recovered	Restless; active	10-15
	b	200	M	Young		Recovered		10-16
	c	200	M	Adult		Recovered	Same rat as a	10-21
	d	300	M	Young		Died	2 minutes; convulsions; 10 minutes dead	10-23
	e	300	F	Pregnant		Died	5 minutes; dead	10-24
	f	400	F	(?)		Died	Convulsions; dead in 30 minutes	10-27
"H"	a	100	M		237	Recovered	1 hour unable to walk	10-15
	b	200	M		141	Recovered	Dopey	10-16
	c	200	M		235	Died	Same rat as a	10-21
	d	200	M		141	Recovered	2 hours convulsions; same rat as a	10-24
	e	200	F		149	Recovered	2 hours convulsions; 18 hours active	10-24
	f	250	M		137	Died	5 minutes twitching; 10 minutes dead; same rat as d	10-27
	g	270	F		150	Died	3 minutes dead	10-23
"H" succinate	a				150	Recovered	{ lying on side and twitching; five minutes after injection	
	b				200	Recovered		
	c				250	Died	Death occurs within 10 minutes	

Minimum fatal dose, intravenous, rabbits. The manufacturers submitted data obtained by the injection of 1 per cent solutions into the ear veins of rabbits, at the rate of 1 cc. per eighteen seconds. These will be cited for comparison.

Minimum fatal dose, hypodermic, rats. Full grown, or nearly full grown white rats were used. The anesthetics were dissolved in $\frac{M}{8}$ NaCl (0.73 per cent). The injections were made in the subcutaneous tissue near the base of the tail. The usual amount injected into each rat was 3 to 4 cc., except in cases of low solubility of the drug, when the amount of the solution was increased just sufficient to bring it into solution. The amount never exceeded 8 cc.

The amount of drug was started at 100 mgm. per kilogram of body weight of the rat injected, and increased by 100 mgm. until the fatal dose was reached. The injections were made on different rats. No surviving animal was injected until at least four days had elapsed after the last injection and symptoms were negative. The minimum fatal doses were all checked by subsequent injections on normal rats. The data are shown in table 4.

Cardiac effects. These were studied by perfusion of turtle hearts, by the technic described in Sollmann's Laboratory Guide, page 198. The heart of the decerebrated turtle was exposed in situ. The inflow cannula was inserted into one vena cava; the outflow cannula was inserted into one aorta; other vessels were tied off. Two perfusion bottles were used: one for plain Ringer's, the other for Ringer's containing the drug. The pressure levels were always maintained constant. The level of the perfusion fluid being about 10 cm. above the heart. The level of output was 5 cm. above the intake level. The apex of the heart was connected with a heart lever which recorded the excursions on the drum of a Harvard kymograph. Outputs were recorded at 2 minute intervals.

The perfusion was conducted as follows:

1. When the heart and apparatus were working satisfactorily, plain Ringer's was perfused for 10 minutes, during which time the (A) excursions, (B) rate, and (C) amount of output were recorded.

2. During the following 10 minutes the solution containing a given percentage of drug was introduced and similar records taken.

3. Plain Ringer's was then resumed for at least ten minutes, or until the heart had returned to normal or to a constant.

4. Then an increased concentration of the drug was introduced and observations made as before. This procedure was continued throughout the series.

From the results obtained the data in table 5 were compiled. For purposes of comparison the percentage concentrations necessary to reduce the excursions and outputs to one fourth or less than the "mean" was arbitrarily taken. The "mean"

TABLE 5

Perfusion of turtle heart

DRUG	PARTS PER 10,000 TO REDUCE		TOXIC RATIO: COCAIN 1	
	Output to $\frac{1}{4}$ or less	Excursions to $\frac{1}{4}$ or less	Output	Excursions
Cocain.....................	$\frac{1}{4}$	$\frac{1}{2}$	1	1
Procain....................	4	8	$\frac{1}{16}$	$\frac{1}{16}$
"G"..........	4	4	$\frac{1}{16}$	$\frac{1}{8}$
"III"......................	$\frac{1}{4}$	$\frac{1}{4}$	1	2
"H".......................	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	1

was an average of the constant before perfusion and the constant after.

In all the experiments, even where the heart was brought to practically a standstill and there was no output as a result of the gradually increased percentage of the drug perfused, the hearts were restored to a constant very near the original by simple perfusion with plain Ringer's with the outlet slightly (4 or 5 cm.) lowered below the intake level.

Direct action on blood-vessels. This was studied by the Lewin-Trendelburg method. This consists in the perfusion of the legs of the pithed frog through the abdominal aorta from a Mariotte bottle. The outflow from the abdominal vein was recorded by a drop-counter; the drug was injected with a syringe into the tube leading to the aorta, using 1 per cent in normal saline.

The detailed technique as followed was that given in Sollman's laboratory Guide in Pharmacology, p. 173.

The results were practically negative for any constricting action on the vessels: the flow remained practically the same.

Vascular synergism with epinephrin. This is an important problem in view of the general practice of adding epinephrin to local anesthetics. It was investigated by the perfusion method, as described in the preceding paragraph. Epinephrin was added to the perfusion fluid, starting with 1:1,000,000; and gradually increasing to maintain an output slightly less than one fourth of the estimated normal. The drop-per-minute output was taken throughout the series. Four, six and eight minute intervals were arbitrarily taken for estimating the output after the drug injection. These times were taken because the six-minute interval coincided very closely with the greatest change of peak in the variation of the output. Plain normal saline was injected at frequent intervals, and a check taken on this in the same way as with the drugs. 1 cc. quantities of 1 per cent solutions of the drugs in normal saline were injected.

The average of the arbitrarily taken intervals (4, 6, 8 minutes) was compared with the average of the output constants determined before and after the injections of the drug. With the output constant expressed in terms of unity the following alterations (average of five series) in output were recorded:

```
Constant............................................... 1.0
Normal saline.......................................... 1.4
Cocain................................................. 1.2
Procain................................................ 3.0
"G".................................................... 2.0
"III".................................................. 3.0
"H".................................................... 4.0
```

Since the normal saline solutions in which the drugs were dissolved, contained no epinephrin the dilutions thus resulting would naturally cause one to expect an increase in the output. This is borne out in the saline injections. The decreased comparative output of cocain may be ascribed to the reputed synergistic actions of cocain and epinephrin. The actions of

the other drugs are distinctly not synergistic. They are antagonistic.

Irritation. This was judged by the conjunctival experiments on human subjects.

Precipitation of albumen. The addition of 1 per cent solutions of "G" or "III," or of $\frac{1}{2}$ per cent solution of "H" did not precipitate a solution of albumen, so that the drugs are not chemically astringent.

TABLE 6

Effective anesthetic concentrations

	"III." ETHYL-AMINO-PROPYL-DIPHENYL-AMINO-CARBINOL HYDRO-CHLORID		"G." AMINO-BENZYL-DIBUTYL-AMINO-ETHANOL HYDRO-CHLORID		"H." AMINO-BENZOYL-DIBUTYL-AMINO-PROPANOL HYDRO-CHLORID OR SUCCI-NATE		PROCAIN. AMINO-BENZOYL-TRIETHYL-AMINO-ETHANOL HYDRO-CHLORID		HOLOCAIN. PHENE-TIDYL-ACETPHEN-ETIDIN HYDRO-CHLORID		COCAIN. HYDRO-CHLORID	
	per cent		per cent		per cent		per cent		per cent		per cent	
Motor nerve, frog, paralyzed in 20 minutes......	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{32}$	$\frac{1}{16}$	$\frac{1}{4}$	$\frac{1}{2}$			$\frac{1}{4}$	$\frac{1}{2}$
Sensory nerve, frog, paralyzed in 20 minutes......	$\frac{1}{2}$	1	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$			$\frac{1}{8}$	$\frac{1}{4}$
Frog skin, anesthesia in 20 minutes.................	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{32}$	$\frac{1}{16}$	$\frac{1}{2}$	1			$\frac{1}{16}$	$\frac{1}{8}$
Rabbit cornea, anesthesia..	1	2	$\frac{1}{2}$	1	$\frac{1}{4}$	$\frac{1}{2}$	4	10	$\frac{1}{5}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$
Human cornea, anesthesia	2*	?	?	1†	?	$\frac{1}{2}$‡	2	?	?	1§	1	2
Intracutaneous anesthesia..			?	$\frac{1}{32}$	$\frac{1}{128}$	$\frac{1}{64}$	$\frac{1}{64}$	$\frac{1}{32}$			$\frac{1}{64}$	$\frac{1}{32}$

* Only partial.

† Good anesthesia about like 1 per cent holocain.

‡ Fair anesthesia, surpassing 1 per cent cocain or eucain, less than 1 per cent holocain.

§ Complete anesthesia

SUMMARY OF RESULTS

The results of the comparative experiments may be presented in tabular form. Tables 6 and 7 show the limit concentrations. In each case, the figure to the left corresponds to the highest concentration that was tried and found ineffective by the method as described; the figure to the right corresponds to the lowest concentration tried that proved effective. Data on cocain, procain (novocain) and phenacain (holocain) are included for comparison.

TABLE 7

Side actions

A. Minimum fatal doses

	"III"	"G"	"H"	PROCAIN	HOLOCAIN	COCAIN
	mgm. per kgm.	*mgm. per kgm.*	*mgm. per kgm.*	*mgm. per kgm.*	*mgm. per kgm.*	*mgm. per kgm.*
Intravenous, cats (determined by Hatcher)	10 15		12.5 15	40 45	10.0	15
Intravenous, rabbits (reported from Abbott Laboratory)	17.5	17.5	12.5			12.5
Hypodermic, cats, Hatcher	50		200 250 hydrochlorid 200 250 succinate	200		60
Hypodermic, white rats, Bonar	400 400	200 300		1000 1250		400 600

B. Heart perfusion

	"III"	"G"	"H"	PROCAIN	HOLOCAIN	COCAIN
Heart, perfused. Turtle concentrations, per 10,000, reducing excursions one-fourth or less	0.25	4	0.5	8		0.5

C. Vascular reactions

Perfusion; epinephrin antagonism (ratio to saline)	2.1	1.4	2.9	2.1		0.9
Conjunctival vessels	Dilated	Dilated	No effect	No effect	Moderately dilated	Blanched

D. Sensory irritation

Human conjunctiva	Very marked burning, persistent irritation	Severe burning 2 minutes, persistent irritation	No irritation	None	Sharp burning 30 seconds persistent irritation	Slight burning, 30 seconds short
Rabbit conjunctiva	Very considerable	Considerable	None	None		None
Intracutaneous	Very severe	Very slight	None	None		None

E. Pupil

Pupil human			Not dilated			Dilated

TABLE 8

Ratio of efficiency and toxicity of anesthetics. Referred to cocain = 1

	"III" ETHYLAMINO PROPYLDIPHENYL AMINOCARBINOL HYDROCHLORID	"G" AMINOBENZOYL DIBUTYLAMINO ETHANOL HYDROCHLORID	"H" AMINOBENZOYL DIBUTYLAMINO PROPANOL HYDROCHLORID OR SUCCINATE	PROCAIN AMINOBENZOYL TRIETHYLAMINO ETHANOL HYDROCHLORID	HOLOCAIN PHENETIDYL ACETOPHENETIDIN HYDROCHLORID	COCAIN HYDROCHLORID
A. Anesthetic efficiency						
Motor nerve paralysis	2	1	8	1		1
Sensory nerve paralysis	$\frac{1}{4}$	2	2	$\frac{1}{2}$		1
Frog skin anesthesia	$\frac{1}{2}$	1	2	$\frac{1}{8}$		1
Corneal anesthesia, rabbit	$\frac{1}{4}$	$\frac{1}{2}$	1	$\frac{1}{5}$	2	1
Corneal anesthesia, human	<1	1+	2+	<1	1+	1
Intracutaneous, human		1+	2	1		1
B. Toxicity						
Toxicity, cat, vein	1	$\frac{2}{3}$	1	$\frac{1}{3}$	$1\frac{1}{2}$	1
Toxicity, rabbit, vein	$\frac{2}{3}$		1			1
Toxicity, cat, hypodermic	$1\frac{1}{5}$			<$\frac{1}{4}$		1
Toxicity, rats, hypodermic	$1\frac{1}{2}$	2	$2\frac{1}{4}$	$\frac{1}{2}$		1
Cardiac perfusion injury	2	$\frac{1}{8}$	1	$\frac{1}{16}$		1
C. Vessels						
Dilator during epinephrin	Dilator 2.1	Dilator 1.4	Dilator 2.9	Dilator 2.1		Constrictor 0.1
Conjunctival	Dilator	Dilator	Nil	Nil		Constrictor
Sensory irritation						
Conjunctival	Very severe	Severe	None	None	Considerable	Slight
Intradermic	Very severe	Very slight	None	None	None	None

Table 6 presents the anesthetic concentrations: table 7 compares the side-actions.

Table 8 shows the ratio of efficiency and toxicity, referred to cocain = 1.

Figure 1 illustrates these ratios by block-graphs. These are arranged by geometric, and not by arithmetic progression, since this corresponds to the progression of concentrations in most of the tests.

CONCLUSIONS

Three synthetic anesthetics were compared with cocain and procain from the standpoint of efficiency and side-actions.

Amino-benzoyl dibutyl amino propanol hydrochlorid ("*H*") was found to produce complete anesthesia on surface application to mucous membranes, such as the conjunctivae, in concentrations about one-half those of cocain, one-tenth those of procain; or about the same as holocain. It has the important advantage of being non-irritant. It does not affect the blood vessels materially, and can be used with epinephrin. On direct application to sensory nerves and on intracutaneous injection, the effective concentrations are about one-half those of cocain or procain; but its toxicity is about the same as that of cocain, or perhaps even somewhat higher; and therefore considerably higher than that of procain.

The compound therefore holds out promise of usefulness for anesthesia of intact mucous membranes, especially in the eye, a field that has not so far been satisfactorily filled by synthetic anesthetics. For injection methods, it has probably no serious advantage over the less toxic procain.

Amino-benzoyl dibutyl amino ethanol hydrochlorid ("*G*") is not quite as effective as the "H," as an anesthetic; and is almost as toxic. It produces considerable irritation which would render it less desirable.

Ethyl-amino-propyl-diphenyl amino carbinol ("*III*") is distinctly less anesthetic than the others; it is about equally toxic, and so much more irritant, that its use would be undesirable.
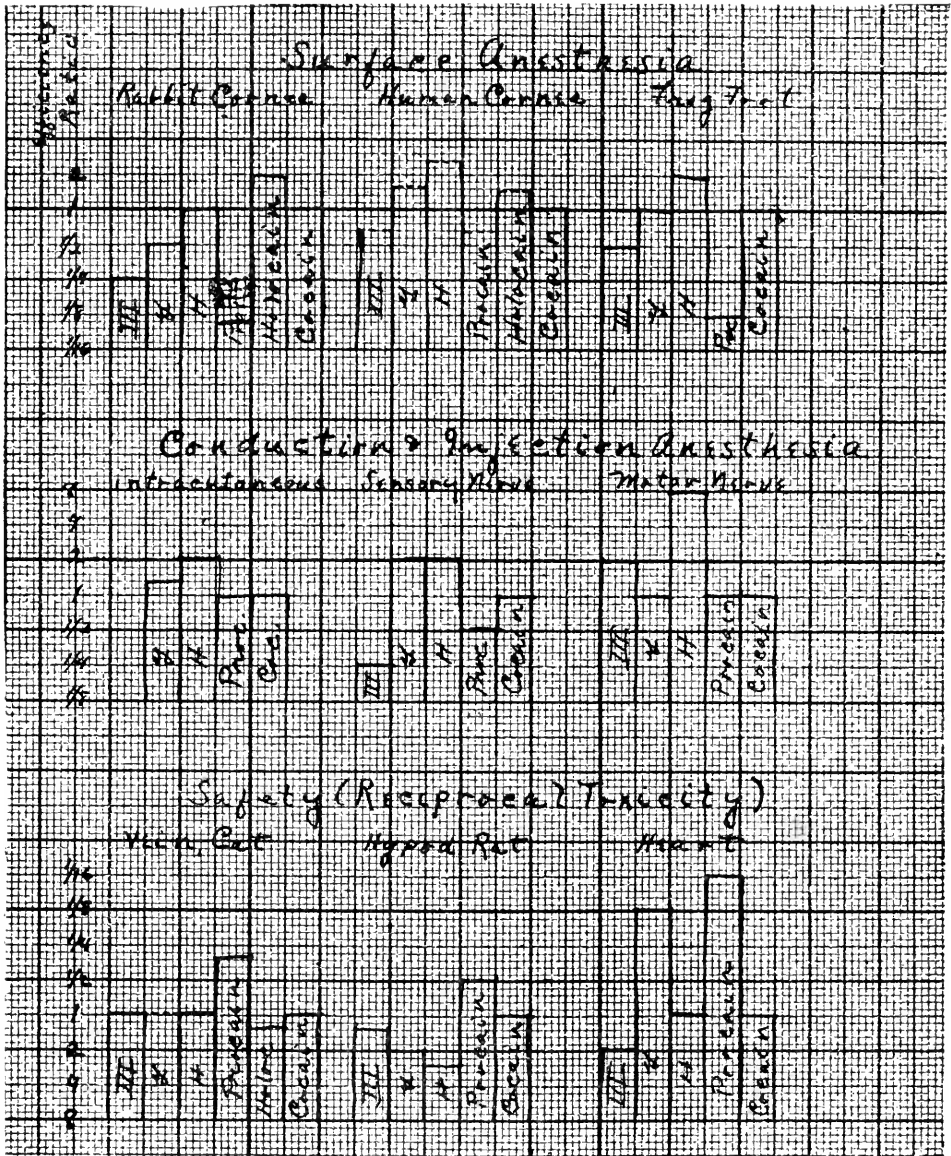
FIG. 1. COMPARISON OF EFFECTIVE AND TOXIC DOSES OF ANESTHETICS

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INDEX TO VOLUME XVIII

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CONTENTS

NUMBER 3, APRIL, 1922

NUMBER 4, MAY, 1922

NUMBER 5, JUNE, 1922

NUMBER 6, JULY, 1922

ILLUSTRATIONS

THE ACTION OF DIPHTHERIA TOXIN UPON THE CIRCULATION

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After the investigations of Romberg, Pässler, Brühns and Müller (1) the collapse of the circulation in infectious diseases became an interesting problem. They examined animals infected with cultures of pneumococci, pyocyanus and diphtheria and found that all these infections had the same effect, paralysis of the vasomotor center, and they considered that this resulted in failure of the heart secondarily. This view is based upon experiments in which they observed the effects on the blood pressure of abdominal massage, aortic compression and irritation of the nasal mucous membrane. Massage of the abdomen, however, was strongly objected to by Von Styskal (2) as insufficient to test the power of the heart. He therefore tested the cardiac efficiency by measuring simultaneously the pressure in the left auricle and in the carotid, while to test the capacity of the vasomotor mechanism the sciatic nerve was stimulated electrically. After ascertaining the reactions in a normal rabbit, he injected a large quantity of a killed culture of diphtheria bacilli. The culture contained 0.1 per cent of carbolic acid and the quantity injected intravenously was as much as 20 to 33 cc. He drew the conclusion from his experiments that the diphtheria toxin acts essentially as a heart poison, the paralysis of the vasomotor center being a secondary result of the weakness of the heart. In his experiments the toxin acted rapidly without any incubation period and antitoxin had no antagonistic effect, both of which are in striking contrast to ordinary experience with these bodies.

1

The contradictory investigations of Romberg and Styskal are the chief contributions to the question of the action of toxins; later, Beck and Slapa (3) showed that there is a direct action on the rabbit's heart, and Fenyvessy (4) on excised frogs' hearts. Bardier also supported this view, while Rolly (5) holds that paralysis of the vasomotor center is the primary cause of the lowering of the blood pressure, and that the heart affection slowly follows.

TABLE 1

Determination of the lethal dose for cats

NUMBER OF CAT	WEIGHT	DOSES OF TOXIN PER KILOGRAM	TIME OF DEATH	REMARKS.
	grams			
1	2400	0.05205	20 hours	Oedema
2	3720	0.01911	44 hours	Local oedema, rise of temperature,
3	2660	0.00470	26 hours	animal restless: post mortem, ec-
4	2710	0.00461	24 hours	chymoses in lungs and lymph glands
5	4020	0.00151	48 hours	here and there
6	2250	0.00111	41 hours	
7	2430	0.00100	48 hours	
8	1780	0.00050	7 days	
9	2660	0.00020	8 days	Local swelling for a few days, rise of
10	2160	0.00019	14 days	temperature, lessened movements,
11	2180	0.00007	7 days	looks slightly ill
Control	Toxin decomposed by heating			
12	3110	0.00402	Recovered	No significant change in the animal's condition

The diverse results of these various workers seem to me to be due chiefly to the mode of intoxication. For example, in Styskal's experiments the toxin and the carbolic acid contained in the injected fluid might have a combined effect which would veil the special effect of the toxin. The carbolic acid should, therefore, be separated from the toxin. The methods of stimulation should be exactly calculable and controlled on normal animals; Romberg and his co-workers made no sufficient measurements.

THE PREPARATION OF THE DIPHTHERIA TOXIN

The most important point in the preparation of the diphtheria toxin lies in obtaining a very strong toxin free from carbolic acid

so that its effect may be revealed with a very small quantity. The killed bouillon culture was evaporated at a low temperature, dried to powder, and then kept in a drying cupboard for three months so that it might be freed from the 0.5 per cent carbolic acid, added at first to the culture to kill the bacilli.   It was then bottled and used after five months.   For use this powder was dissolved in saline in the proportion of 1:100.   Its toxicity for the cat when given by hypodermic injection is shown in table 1.

The cat dies within two days after the subcutaneous injection of 1 mgm. per kilogram of body weight.   As the control shows, the 0.5 per cent Witte's peptone and other substances contained in the culture medium (Bouillon) have no toxic effect.

METHODS OF EXPERIMENTS

The cats were decerebrated under ether with Sherrington's decerebrator, a tracheal cannula being inserted for use in case of the stoppage of respiration.   The blood pressure in the carotid was measured with a mercury manometer.   A tracing of the respiration was made by a modified tambour placed on the chest wall.   The irritability of the vasomotor center was estimated by stimulation of the central end of a divided sciatic nerve by shocks from a secondary coil, the distance from the primary being noted.   When some weakness in the vasomotor center was shown by this way, asphyxia was induced to find whether the spinal vasomotor centers were involved.   The conducting capacity of the vasomotor nerves was tested by determining the minimal stimulus of the splanchnic nerve necessary to increase the blood pressure; this was done in normal and poisoned cats, the conditions being kept the same in each as far as possible.   The condition of the heart was tested by observing the rise of blood pressure when the aorta was compressed by means of Condon's clamp (6) placed on the aorta directly below the left renal artery; in normal cats, closure of the clamp is followed immediately by a rise of blood pressure, while in the exhausted heart no significant increase in the arterial tension is observed.   All these methods were applied first to

TABLE 2

EXPERIMENT	WEIGHT OF CAT	INJECTION	TEMPERATURE		DECEREBRATION	HEART STOPPAGE ON RECORD	STOPPAGE OF RESPIRATION
			Before injection	Before decerebration			
	grams		°C.	°C.			
1	2100	Normal bouillon 0.5 cc. per kilogram subcutaneously 18 hours before operation	37.0	37.0	11.00 a.m.	6.20 p.m.	6.56 p.m.
2	2970	Normal bouillon 0.6 cc. per kilogram subcutaneously 24 hours before operation	37.0	37.0	10.15	6.10	7.14
3	2400	Toxin 0.0520 gram per kilogram intraperitoneally, 1 hour after operation	37.0	3 hours later	11.10	5.10	4.45
	2400	Toxin 0.0520 gram per kilogram intraperitoneally, 2 hours after operation		38.5			
4	2350	Toxin 0.0532 gram per kilogram intraperitoneally, 4 hours before operation	36.9	37.2 (39.9° 2 hours later)	2.40 p.m.	4.35	4.30

					11.10 p.m.	11.59	
5	2760	Toxin 0.0015 gram per kilogram subcutaneously 23 hours before operation	37.5	39.5			The respiration stopped after operation. Artificial respiration applied
6	4020	Toxin 0.0015 gram per kilogram subcutaneously 47 hours before operation	37.5	35.0	11.00	12.50	The respiration stopped directly after the operation, and recovered slowly after artificial respiration in the form of Cheyne-Stokes respiration, which stopped at 12.50

normal cats, and then to injected ones at various stages of the intoxication.

THE GENERAL COURSE OF CONTROL AND OF INTOXICATED CATS AFTER DECEREBRATION

As controls two cats were injected subcutaneously in the neck with 1.2 cc. of normal bouillon. After eighteen or twenty-four hours their condition was quite normal without any rise of temperature in the rectum. They were then decerebrated under ether and the respiratory movements and blood pressure were recorded every half hour, the animals being kept warm on a copper box containing warm water. The temperature in the rectum was noted at the time of tracing. Blood pressure and temperature became gradually lower, until the pulsations of the manometer were so weak that they could hardly be made out and finally disappeared, whilst the respiration though a little irregular still continued. The irregularity of the respiratory movements increased until they also ceased. The stoppage of the pulse on the manometer tracing occurred eight or nine hours after decerebration whilst the respiration continued for about an hour longer. The final arrest of the heart must have occurred at the same time as that of the respiration, that is, about nine to ten hours after decerebration.

Four cats were observed in the same way after injection of diphtheria toxin; the quantity of toxin, the mode of its injection, and the lapse of time after the injection were modified in each case in order to see what difference would arise from variations in the intensity of the intoxication (see table 2).

In all four cases of poisoning the general features are the same and differ from the control chiefly in that after a latent period the cat became fevered and that it could not be kept alive as long as the control even when the injection was made after the decerebration and that the failure of the respiration occurred while the pulse was still visible in the blood pressure tracing; the cessation of respiration is sometimes preceded by typical Cheyne-Stokes breathing. Two protocols may be given as illustrations.

TABLE 3

TIME	TEMPERATURE	RESPIRATION	BLOOD PRESSURE
			mm.
10.15 a.m.	37.0	3S	SS
		Regular	
10.45 a.m.	34.5	34	S4
		Regular	
11.30 a.m.	34.5	34	S0
12.0 m.	34.5	36	64
12.30 p.m.	34.7	36	60
1.0 p.m.	35	32	70
1.45 p.m.	34.7	42	70
		A little irregular	
2.0 p.m.	34.7	42	44
		A little irregular	
2.30 p.m.	34.S	36	55
		Regular	
3.0 p.m.	34.S	32	60
		Regular	
3.30 p.m.	34.S	32	5S
		Regular	
4.0 p.m.	34.S	34	64
		Regular	
4.30 p.m.	34.S	3S	55
		A little irregular	
5.0 p.m.	33.5	32	55
5.30 p.m.	32.S	35	50
		Regular	
6.0 p.m.	32.S	30	50*
6.15 p.m.	32.S	16	26
		Irregular	
6.50 p.m.	30.0	S	20
		Irregular	No pulse visible on tracing
7 14 p.m.	30.0	Respiration failed	0

* Very slight pulse visible in carotid.

Experiment II. Cat 2

October .15, 1920. 10.15 a.m. Weight 2975 grams, temperature 37°. 1.8 cc. normal bouillon was injected subcutaneously into neck.

October 16, 1920. 10.15 a.m. Temperature 37°. No change in condition, decerebrated under ether, the respiration and blood pressure recorded every half hour until death, as shown in table 3.

Experiment VI. Cat 6

October 9, 1920. 11.00 a.m. Weight 4020 grams, temperature 37.5°. Diphtheria toxin 0.0015 gram per kilogram was injected subcutaneously into neck.

October 10, 1920. 10.00 a.m. Animal looked slightly ill, temperature 38.0°; very still; some stiffness about neck.

October 11, 1920. 10.00 a.m. Very sick; lay on its belly, temperature 35.0°. Decerebrated under ether. Directly after decerebration the respiration became very weak and artificial respiration was begun until the spontaneous breathing returned. Soon afterwards it changed to Cheyne-Stokes form and ceased after twenty-five minutes. The pulse though weak, was regular and lasted for a few minutes after the respiration (see table 4).

TABLE 4

TIME	RESPIRATION	BLOOD PRESSURE
11.45	4	54
11.52	Artificial	44
11.54	12	
	Spontaneous Cheyne-Stokes	48
11.59	1	48
12.3	0	68
12.4	24	54
	Irregular	
12.5	0	60
12.37	4	26
12.41	Respiration stopped	12

EXPERIMENTS UPON THE INCUBATION TIME

In previous experiments I found that many hours of incubation elapsed before the appearance of the fever which is the first symptom. As, however, some workers quoted above insist on the immediate action of the toxin upon the heart, the four following experiments were carried out. During the experiments with records of the respiration, blood pressure and temperature, two cats were injected intraperitoneally, the other two intravenously with a very large dose of toxin, and the tracing continued until death. At whatever stage of the tracing the toxin was injected, no immediate effect was observed upon the blood

pressure, heart beat or temperature, even when the quantity of toxin injected was as much as one hundred times the lethal dose.

The latent period continued for 3 to 5 hours, according to the quantity injected, and then the temperature fell and the blood pressure fell somewhat while the respiration became weaker and slower. This was probably attributable to the operation, because a similar change in the blood pressure, breathing and temperature after some time was observed in the control also. As an exam-

TABLE 5

TIME	BLOOD PRESSURE	RESPIRATION
11.2	104	29
11.3*	104	29
11.5	104	30
11.35	105	32
12.0*	100	27
12.1	102	27
12.3	102	29
12.5	105	29
12.13	100	28
12.30	90	29
1.0	89	29
1.45	86	29
2.0	88	29
2.24	58	25
3.7	20†	14
3.20	18	9
3.35	16	4
3.43	14	Respiration ceased

* Seventeen minimal lethal doses injected into jugular vein.
† No pulsation visible. Respiration irregular.

ple of the action experiment 9 may be cited, in which 34 lethal doses of the toxin were injected intravenously in a decerebrated cat of 2420 grams weight.

ANALYSIS OF THE LOWERING OF THE BLOOD PRESSURE

Compression of the aorta and stimulation of the central end of the sciatic nerve were used to find whether the heart itself or the vasomotor center was affected by the toxin.

Four control cats were examined in order to test these methods and the thresholds of stimulation of the nerve were determined.

One of the controls was injected intravenously with 10 cc. normal bouillon and the threshold was carefully determined at intervals before and after the injection until the death of the animal. Six cats were injected subcutaneously with small or large lethal doses and after symptoms of poisoning developed were examined under the same conditions as the controls.

In these ten experiments I could detect no difference in the rise of blood pressure from aortic compression between the control and the intoxicated animals; in both sets the blood pressure rose immediately. The reaction gradually weakened throughout the course of the experiments until finally there was no rise a few minutes before death. The vasomotor reaction on sciatic stimulation was different in the control and intoxicated cats. The threshold strength of stimulus in the controls soon after decerebration was at 17 to 30 cm. whilst that of the poisoned animals was about 10 to 13 cm. In both the control and the intoxicated cats the threshold decreased gradually with the lowering of the blood pressure, and finally no rise of the blood pressure was observed from strong stimulation. This diminution of the irritability took place in the intoxicated cases more quickly and to a greater degree than in the control. These observations indicate that the vasomotor system is less readily aroused to activity after the symptoms begin from diphtheria toxin; the reaction in these poisoned animals immediately after decerebration is the same as that of controls which have been exposed for several hours after decerebration, and the debility of the vascular control progresses even more rapidly than in the controls.

In one experiment, the effect of sciatic stimulation was examined in an unpoisoned cat, and then 63 minimal lethal doses of toxin were injected intravenously and the stimulation was repeated at intervals for an hour. No change was observed in the threshold stimulus or in its effects during this time, this being in accord with the other observations already recorded that the effects of the toxin only appear after a long latent period.

Experiments were then carried out by splanchnic stimulation to find whether the peripheral vasomotor nerves or the medullary center is the site of the action of the toxin. Control

experiments were performed on normal cats and these were compared with cats injected with toxin eighteen to twenty-four hours previously. Artificial respiration was employed, and the splanchnic nerve was exposed by Sherrington's method, and the electrodes applied to its distal stump.

The effect of splanchnic stimulation seemed to be less when the blood pressure was low, but in this respect no difference could be detected between the poisoned cats and the controls. So that the conduction of the peripheral vasomotor nerves and their terminations appears to be little if at all affected by the toxin. And as no evidence was obtained of weakness of the heart in the experiments in which the aorta was compressed, while those in which the sciatic nerve was stimulated indicated some reduction in the activity of the vasomotor centers, the reduction in the blood pressure appears to arise for the most part from failure of that center.

SUMMARY

The effects of diphtheria toxin on the circulation and respiration only appear many hours after its injection even when a dose that is many times that ultimately fatal is injected intravenously. All attempts to analyse its action in acute experiments are therefore futile, and further light can be thrown on its effects only by examining the condition of animals subjected to it many hours previously and comparing their symptoms with those of controls. In a series of such experiments, the blood pressure was found to be lower than in the controls and this appeared to be due to failure of the central vasomotor mechanism. No evidence of direct action on the peripheral vasoconstrictor nerves, or on the vessels of the heart, was obtained.

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STUDIES ON STIMULATION OF THE RESPIRATION: THE ACTION OF RESPIRATORY STIMULANTS UPON THE RESPIRATION WHEN DEPRESSED BY INCREASED INTRACRANIAL PRESSURE WITH SPECIAL REFERENCE TO SODIUM CYANIDE

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The work herein presented was suggested by our observing clinically a case of increased intracranial pressure due, as the autopsy proved, to severe cerebral edema in which the respiration became depressed and the problem of selecting a suitable respiratory stimulant presented itself. The absence of any experimental study of the best method of stimulating the respiration in this condition was strikingly brought home to us and it was determined to make a comparative study of the value of respiratory stimulants which have been proposed from time to time, in order to determine which is the best under the stated condition. It is obvious that, as our detailed knowledge of drugs increases, we shall not speak of respiratory stimulants in general, because a substance which stimulates the respiration when this is depressed by one means will not necessarily stimulate it when depressed by other means. In order, therefore, to stimulate the respiration efficiently, we shall have to know or form some idea of the cause of the respiratory depression since the best respiratory stimulants may vary according to the cause of the depression. In most of the experimental work that has been done, the effect of drugs has been studied on the normal respiratory center, which is an entirely different proposition from the respiratory center when depressed by various means These remarks also apply more or less to stimulants and depressants in general.

13

The effect of increased intracranial pressure on the circulation, respiration and other associated phenomena has been the subject of many physiological and clinical investigations (1). The method of treating cases of increased intracranial pressure by drugs has received very little attention, the main efforts having been directed toward the perfecting of surgical technic of performing decompression operations, but, in many cases, as the cerebral edema associated with acute infections, cerebral hemorrhage, and other conditions, decompression is rarely considered. In traumatic cases with increase in intracranial pressure, the respiration may be so seriously embarrassed that immediate stimulation of the respiration is indicated and this must be continued during the operation for decompression. Furthermore, it has been shown by many investigators that effects, quite similar to those of increased intracranial pressure, can be produced by decreasing the blood supply to the brain by other means than increasing the intracranial pressure. In fact, the effects on the circulation and respiration of asphyxiation however produced closely simulate those of increased intracranial pressure. It would seem probable that the same therapeutic considerations which apply to increased intracranial pressure would also apply to other conditions of cerebral anemia. Previous workers on intracranial pressure have described the general phenomena associated therewith in the circulation and respiration and it will be unnecessary for us to go into this phase of the subject except in so far as it is essential in describing our own experiments.

METHODS

Dogs were used exclusively in this investigation. Ether was employed as the anesthetic. The dogs were trephined under complete surgical anesthesia, the dura excised beneath the opening and a cerebral cannula introduced. This was connected with the pressure bottle. The pressure bottle contained Ringer's solution at body temperature. A mercury manometer was inserted in the intracranial system. The intracranial pressure was recorded by means of a float on the mercury manometer. The

blood pressure was taken from the carotid. The zero blood pressure and zero pressure of the intracranial system were recorded on the same line on the kymograph so that the relation of the intracranial pressure to the blood pressure can be seen at a glance. In order to measure accurately the lung ventilation, the respiration was recorded by the following means: The dog was placed on a specially constructed board to fit into a body plethysmograph built on the same principle as that described by Haldane and Priestley (2). The plethysmograph was connected with a large counterpoised piston recorder. This piston recorder was calibrated so that it was possible to calculate the actual ventilation of the lungs from the record. The movements of the piston recorder were registered on the kymograph by means of a pointer attached to it. The opening around the dog's neck was made tight by means of the small inner tube of a bicycle tire, which fitted into a groove in the plethysmograph. The tube was inflated to such a point as to prevent the passage of air to or from the box, but not to the point of compressing the veins of the neck. The apparatus is very simple and satisfactory but the piston recorder must be kept scrupulously clean as it works without lubrication.

The experiments which we have done fall into two classes:

1. Those in which a constant relation was maintained automatically between the blood pressure and intracranial pressure: Part 1.

2. Those in which the intracranial pressure was held at a constant level and did not alter with changes in the blood pressure: Part 2.

PART 1. THE EFFECT OF VARIOUS DRUGS ON THE RESPIRATION WHEN IT IS DEPRESSED BY A CONSTANT GRADE OF MEDULLARY ANEMIA

The first set of experiments was devised in order to determine the effect of the drug being studied on the respiration independent of blood pressure changes. This was done by means of the device described below, by which a rise or fall of the arterial

blood pressure caused a corresponding rise or fall of the intra-
cranial pressure so that a constant grade of cerebral anemia
was maintained regardless of the response of the circulation
to the drug administered.   The arrangement is shown in figure 1.

The Kolls valve, figure 1, D, has been figured and its
operations described by A. C. Kolls (3).

The operation of this apparatus may be described as follows:
When the float P makes contact between the mercury in manom-
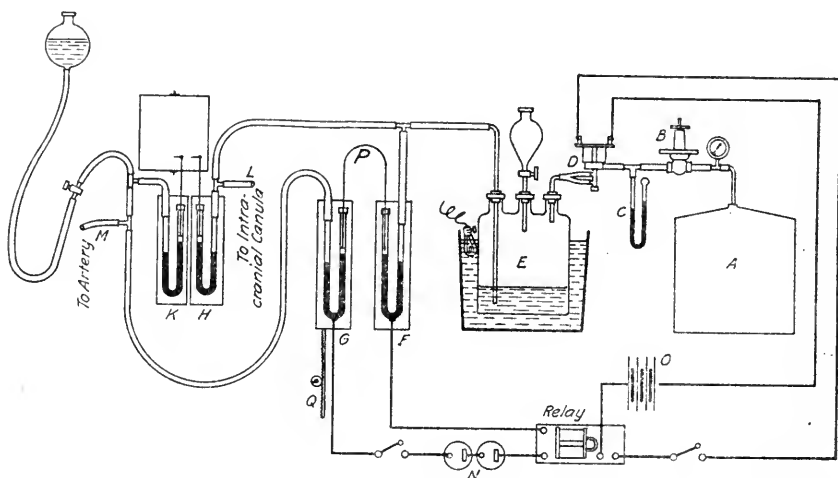
FIG. 1. A, air pressure tank; B, reducing valve; C, manometer; D, Kolls
valve; E, Ringer's solution; F, accessory intracranial pressure (control) manom-
eter, platinum wire sealed in at the bottom; G, accessory blood pressure (con-
trol) manometer, platinum wire sealed in at·the bottom; H, manometer recording
intracranial pressure; K, manometer recording blood pressure; L, tube to intra-
cranial cannula; M, tube to arterial cannula; N, two dry cells; O, storage batteries,
P, float making electrical connection between manometers G and F; Q, rachet
by means of which the accessory blood pressure manometer can be raised or
lowered.

eters F and G, the dry cell circuit is closed, the magnets of the
relay are actuated and the battery circuit actuating the magnet
of the Kolls valve (D) is broken so that there is an escape of air
from E through the valve, resulting in a lowering of the intra-
cranial pressure.   The instant the intracranial pressure falls
sufficiently so the contact is broken between the float P and the

mercury in manometer F, air flows into E until the contact is restored. When, due to a rise of blood pressure the float P is raised so that connection is broken between F and G, the battery circuit is closed and air flows from the tank A into E resulting in a rise of intracranial pressure which continues until contact is again made with the float P when the air again begins to escape from E. In actual use the valve D is constantly operating in one direction or the other and we have been able except in cases of very rapid changes in the blood pressure, to maintain a constant grade of cerebral anemia.

Figure 2 is a tracing which illustrates how closely changes in the blood pressure are followed by changes in the intracranial pressure. By means of the rachet Q the relative positions of manometers F and G could be controlled and any desired relation can be established and maintained between the blood pressure and the intracranial pressure. Thus for example the manometers may be set so that the intracranial pressure will be maintained at a point 5 mm. below the blood pressure. The method of procedure in each experiment was to increase the intracranial pressure more or less gradually until the respiration was definitely depressed. The intracranial pressure was then reduced to zero, and the respiration and circulation allowed to return to normal. The intracranial pressure was then increased a second and a third time more rapidly to the same level that had previously depressed the respiration in order to make certain of the effect. On the third or fourth trial the drug was injected while the respiration was depressed and its effect noted. After observing the effect of the drug administered, the intracranial pressure was again reduced to zero. When the respiration and circulation had again returned to normal, the intracranial pressure was increased to a higher level than in the first set of observations and in this way the effect of the drug could be determined under various degrees of anemia of the medulla.

The drugs studied include: Sodium cyanide, strychnine sulphate, atropine sulphate, caffeine citrate, and lactic acid.
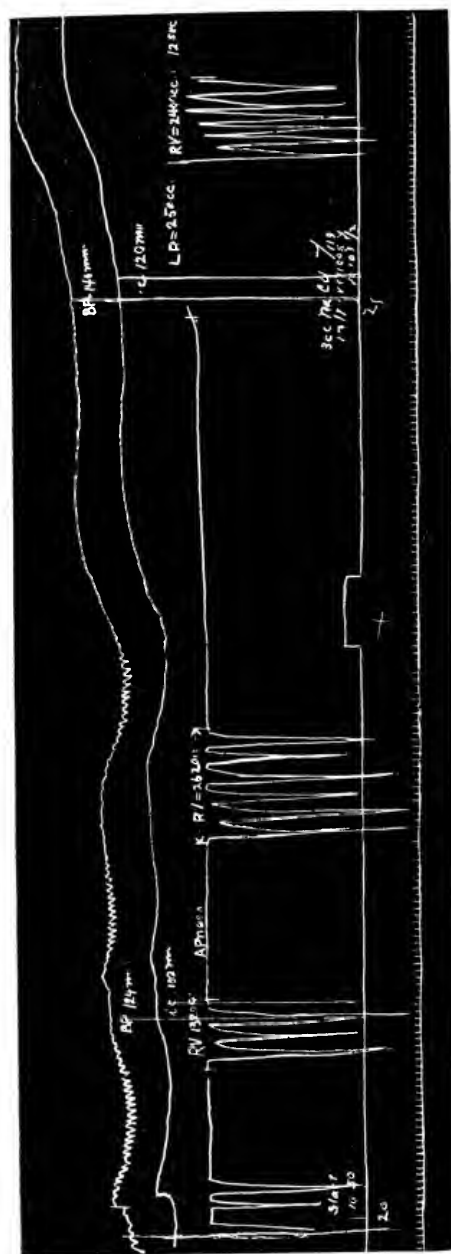
Fig. 2. Upper tracing, carotid blood pressure. Second tracing, intracranial pressure. Third tracing, respiratory record. Fourth tracing, base line for intracranial pressure and blood pressure. Fifth tracing, time record in seconds.

The effect of sodium cyanide on the respiration when depressed by a constant grade of medullary anemia

In this work the sodium cyanide was given in 0.01N solution in 0.9 per cent sodium chloride. All injections were made into the jugular vein. Sodium cyanide cannot be used satisfactorily in any other way than by intravenous injection. It is the only safe way of administering the drug. Hundreds of intravenous injections of the therapeutic doses of sodium cyanide have been made by us in animals and man (4) without a single accident or untoward symptom. When given by any other route, absorption is so uncertain that one obtains either little or no effect whatever or more or less severe poisoning results. When injected intravenously, in therapeutic doses in dogs, the stimulation of the respiration begins in 8 to 25 seconds. The height of action of the drug occurs ordinarily within 25 seconds and the entire effect is usually over within 60 seconds after ceasing the administration of the drug. The rapidity with which the cyanide acts and the brevity of the period of stimulation following a single injection are shown in figure 6.

The power of cyanide to interrupt a long period of paralysis of the respiration and reinstate the activity of the respiratory center is shown in figure 3.

These brief periods of stimulation can be repeated any number of times. It only occurred to us after this part of the work was completed that the cyanide should have been given slowly and continuously in some of these experiments. This was only done later in our experiments in which the intracranial pressure was held constant and did not vary with the blood pressure. The evidence is very strong, however, that, by the slow continuous intravenous injection of the cyanide, continuous stimulation of the respiration would have resulted even in these experiments with the constant grade of medullary anemia.

The fact that the cyanide retains its power to stimulate the respiratory center almost up to the moment of death is illustrated in figure 4.

Here the cyanide markedly stimulates the center when the blood pressure has fallen to 39 mm. The animal had inter-
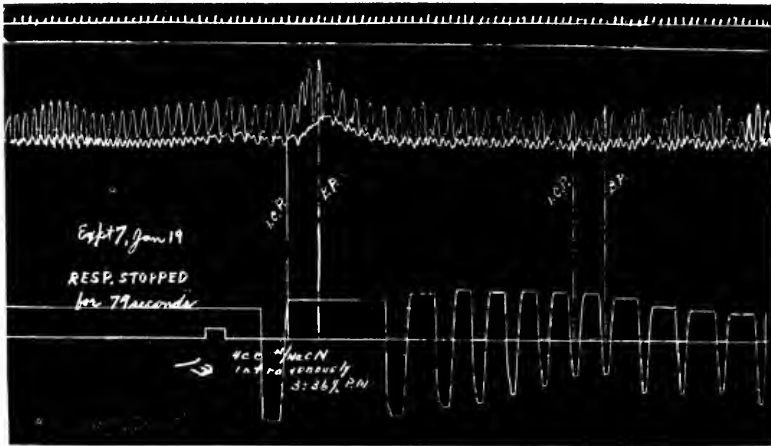
FIG. 3. Fourth tracing from bottom, carotid blood pressure. Third tracing, intracranial pressure. Tracing next to base line, respiratory record.
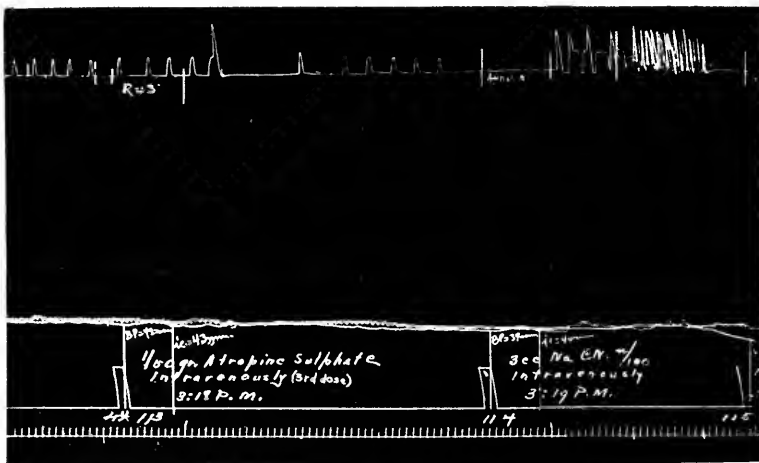
FIG. 4. Upper tracing, respiratory record. Next two tracings, nearly super-imposed, are respectively, blood pressure and intracranial pressure.

mittently been subjected to increased intracranial pressure for $5\frac{1}{2}$ hours at the time this record was taken. The cyanide here stimulated the respiration up to within 2 minutes of death. It is

also of interest to note in figure 5 that the injection of 1.3 mgm. of atropine sulphate failed to cause any stimulation of the respiration. The effect of atropine will be discussed later.

The conclusions from this part of the work are manifest:

1. Sodium cyanide is a very efficient stimulant to the respiration when depressed by anemia of the center.

2. It exercises its stimulating action on the respiratory center directly and acts independently of any change in the circulation, because, regardless of any change in the circulation, a constant
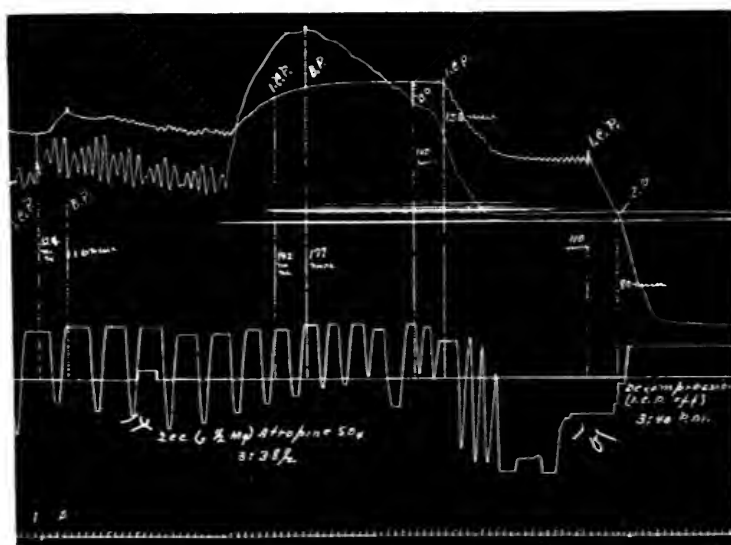
Fig. 5. Upper tracing, intracranial pressure.   Second tracing, blood pressure. Third tracing, respiratory record.

grade of anemia was maintained throughout these experiments. In many cases the injection of the cyanide caused changes in the blood pressure, but usually no significant change in the blood pressure followed its administration. When any effect on the blood pressure was manifest, it usually was a rise of blood pressure but in some cases a fall of blood pressure occurred. The effects of single injections on the respiration and blood pressure were usually of less than one minute's duration. The dosage of cyanide here used did not cause any change in the pulse rate.

The effect of strychnine sulphate on the respiration when depressed
by a constant grade of medullary anemia

Two experiments only were done in this connection. The
dosage used was 0.125 mgm. intravenously in each case. In
one of the animals this dose was given when the increased intra-
cranial pressure was raised to 20 mm below the blood pressure
and had caused the following departures from the normal:

	RATE	VENTILATION	PULSE RATE
Respiration normal......	36	1020	126
Under increased intracranial pressure....	12	840	42

Maintaining the same degree of intracranial pressure, the
injection of the strychnine brought about the following changes:

RESPIRATORY RATE	VENTILATION	PULSE RATE
30	1770	60

The blood pressure rose from 129 mm. before the strychnine to
140 mm. but as the intracranial pressure went up correspond-
ingly there was no change in the blood supply to the medullary
centers. The improvement of the respiration as a result of the
strychnine must be attributed to the action of the strychnine on
the center. Three minutes later the dose was again repeated.
The stimulating effect of the strychnine on the respiration
lasted a long time, certainly 30 minutes. The animal showed
no evidence of strychnine poisoning, neither tremors nor con-
vulsions.

In the second experiment the proof of the stimulating action
of the strychnine was not so conclusive but here also the stimu-
lating action of the drug was seen. The animal withstood
usually high intracranial pressure before succumbing.

We conclude from these experiments that strychnine directly
stimulates the respiratory center when depressed by a constant
grade anemia due to increased intracranial pressure. The
stimulation lasts much longer than that of sodium cyanide.

The duration of stimulation may be put conservatively at twenty to sixty minutes. From a larger number of experiments with strychnine sulphate to be referred to later, we may add that the beneficial action of strychnine on the respiration is not seen in every case.

The effect of atropine sulphate on the respiration when depressed by a constant grade of medullary anemia

Three experiments were performed with atropine sulphate under the condition stated. The dosage used varied between 0.5 mgm. and 1.3 mgm. in each case.

The results were as follows:

Experiment 5. Before atropine the respiratory rate was 6 per minute and the lung ventilation was 2550 cc. per minute. Within fifteen seconds after the atropine was given the respiration increased in rate to 30 per minute and decreased in depth, but the total ventilation increased about 150 per cent, namely from 2550 to 6300 cc. per minute. The stimulation lasted four minutes when the respiration ceased. The heart continued to beat about 30 seconds after the last respiration. Following the administration of the atropine, the blood pressure rose from 94 to 106 mm., but after 3.5 minutes, the blood pressure began to fall rapidly and continuously until death occurred.

Atropine undoubtedly hastened the death of the animal.

Experiment 6. In this experiment the atropine caused a rise of blood pressure from 145 mm. to 193 mm., the rise occurring so precipitously that the intracranial pressure could not follow the blood pressure accurately with the result that there was an increase in the blood supply to the respiratory center. In spite of the improvement in the circulation the respiration which was greatly depressed (4 per minute) showed no stimulation whatever.

Experiment 7. The portion of the record showing the effect of the injection of 0.5 mgm. of atropine sulphate are shown in figure 5. Here the blood pressure rose in twenty-two seconds after the drug was given from 124 to 177 mm., then fell continuously until death, which occurred within three minutes. The respiration showed very slight stimulation for about one minute following the administration. There can be no doubt that the atropine was responsible for the sudden death of this animal.

We conclude therefore, that, in medullary anemia due to increased intracranial pressure, atropine sulphate is a very uncertain respiratory stimulant. It either had no effect on the respiration in our experiments or it caused a stimulation of very brief duration. As a therapeutic measure its administration is worse than useless. In two of the three experiments, it greatly hastened the death of the animal and in the remaining experiment it was without any beneficial effect whatever.

The effect of caffeine citrate on the respiration when depressed by a constant grade of medullary anemia

Three experiments were carried out with caffeine citrate, using doses of 16 to 32 mgm.

In the first experiment the injection of 32 mgm. had no effect on the respiration whatever. In the second experiment, the same dose caused marked stimulation of the respiration lasting about 6 minutes. About three minutes after the injection, the blood pressure began to fall and continued downward until death which occurred in less than 10 minutes after the injection. A second injection of 32 mgm. three minutes before death had no effect whatever. In the third experiment the injection of 16 mgm. during paralysis of the respiration reinstated the respiration 30 seconds after the administration of the drug. The respirations were 24 per minute and the ventilation of the lungs 5160 cc. per minute, against the normal before intracranial pressure was applied of 78 per minute and a ventilation of 3900 cc. After three minutes the blood pressure began to fall and the respiration stopped permanently, death occurring 11.5 minutes after the drug was administered. A second dose of 16 mgm. given when the respiration had failed, produced no effect whatever.

We conclude from these experiments that although caffeine citrite causes a brief but definite stimulation of the respiration, its effect is harmful rather than beneficial. Its harmful effect may be primarily on the circulation which shows signs of failure shortly before respiration.

The effect of lactic acid on the respiration when depressed by a constant grade of medullary anemia

Two experiments were performed with lactic acid. The acid employed was the racemic acid (Kahlbaum) and was used in a dosage of 1 cc. of 0.1N solution injected intravenously either undiluted or diluted with 1 or 2 cc. of 0.9 per cent salt solution.

In the first experiment the acid was given when the intra-cranial pressure was very slightly below the blood pressure and the respiration was greatly depressed, the rate being 5 per minute. Within about 75 seconds the respiratory rate had increased to 13 per minute without any change in depth, the inspirations being about 200 cc. before and after the drug. This increased rate continued with gradually diminishing amplitude until the respiration failed 11.5 minutes after the first injection.

A second injection of 1 cc. was given 3 minutes after the first one. It gave only a slight stimulation and a third injection given at the time the respiration failed gave no response whatever. After paralysis of the respiration for 70 seconds the animal gave a few gasps and died 26 minutes after the first dose of lactic acid was given.

In the second experiment, with the intracranial pressure and the blood pressure practically equal and the respiration paralysed, lactic acid failed to stimulate the respiration as did also sodium cyanide at this time. After decompression, the respiration returned. On again raising the intracranial pressure to a point 12 mm. below the blood pressure, the respiration again became greatly depressed, in fact it was practically nil, and 1 cc. 0.1N lactic acid was again given. This reinstated regular respiration at the rate of 18 per minute. The respirations were of great amplitude—average 225 cc. per respiration, so that the ventilation per minute was 4050 cc. against the normal for this animal before applying intracranial pressure of 3210 cc. with a rate of 102 per minute. This stimulation of the respiration with a gradual rise of blood pressure from 84 mm. to 114 mm. continued for 14 minutes when vomiting efforts by the animal brought up a small amount of mucus. After a lapse of 10 minutes, the

blood pressure began to fall rather suddenly to 77 mm. so that the animal was decompressed. On again raising the intracranial pressure to approximately the height of the blood pressure, the respiration became embarrassed within 3 minutes when 1 cc. of lactic acid was given again. After a period of 1.5 minutes regular and vigorous respirations returned, having a rate of 42 per minute, but the blood pressure was falling gradually and continuously and the respirations finally ceased 6 minutes after the lactic acid was given.

We conclude from these experiments that independently of blood pressure changes, lactic acid in certain animals is an efficient stimulus to the respiration when depressed by increased intracranial pressure. However, a larger series of experiments to be presented later show that lactic acid is very unreliable as a respiratory stimulant. It would seem to have a deleterious effect on the circulation in the dosage here used.

We may summarize the work of part 1 as follows:

1. Sodium cyanide stimulates the respiration independently of blood pressure changes. The response is a matter of seconds and stimulation in most animals follows its administration almost up to the time of death. It is the most certain and dependable stimulant to the respiration, when depressed by increased intracranial pressure, with which we have worked. The dosage required to stimulate the respiration is well below the toxic dose and no untoward effects from its administration were noted. The stimulation lasts but a brief period of time, usually less than one minute.

2. Strychnine sulphate also stimulates the respiration, when depressed by increased intracranial pressure, independently of blood pressure changes. The stimulation lasts much longer than that caused by sodium cyanide. It often continues from 20 to 60 minutes. Stimulation of the respiration does not always follow the giving of strychnine. In some animals, it was a total failure as a respiratory stimulant.

3. Atropine sulphate is a very uncertain stimulant under the given conditions. It either has no effect on the respiration or causes a stimulation of very brief duration. In regard to pro-

longing the life of the animal, it is either worthless or harmful. In most cases it hastens the death of the animal.

4. Caffeine citrate, under the conditions stated, causes a brief stimulation, but its effect is harmful rather than beneficial. It probably exercises a deleterious effect on circulation since the latter usually fails before the respiration.

5. Lactic acid in doses of 1 cc. of 0.1 N solution is an efficient stimulus to the respiration, but the dosage is not as capable of being controlled as sodium cyanide.

PART 2. THE EFFECT OF VARIOUS RESPIRATORY STIMULANTS WHEN THE RESPIRATION IS DEPRESSED BY A CONSTANT GRADE OF INTRACRANIAL PRESSURE

In part 1 of this paper, we desired to determine whether certain substances stimulate the respiration directly or whether they act on the respiration only indirectly by improving the circulation through the medulla. In order to determine this point definitely, we used the apparatus already described which prevented any change in the blood pressure from altering the blood supply to the medulla. By this method of study, the drug was placed under a great disadvantage from the therapeutic standpoint since an improvement in the respiration might cause an improvement in the circulation as well and this latter effect would augment the therapeutic efficiency of the drug. In the experiments under consideration, as well as in the therapeutic application of the results, the complete interdependence of the respiration and circulation must be borne in mind constantly. The object of the experiments in part 1 was simply to determine the factors in any stimulating effect that the drug might exhibit. It is obvious that the experiments in which a constant relation between blood pressure and intracranial pressure was maintained could not have the importance from a practical therapeutic standpoint comparable to experiments in which the intracranial pressure was held constant as is the case clinically. For this reason, very much fewer experiments were performed in part 1 than in part 2.

In the experiments which follow the intracranial pressure was increased gradually until the respiration showed stimulation. The intracranial pressure was then increased more cautiously about 3 to 5 mm. at each increment until the respiration was greatly depressed or paralyzed. The intracranial pressure was then reduced to zero and after conditions returned to normal, the intracranial pressure was again rapidly raised to the point which had been previously found to embarrass the respiration. This was repeated from 3 to 4 times until it was certain that the critical point of intracranial pressure had been determined. Finally this height of intracranial pressure was mantained and with the respiration depressed, the drug was administered. The animals required little or no anaesthetic after the application of intracranial pressure, since this caused loss of consciousness. Variation in the depth of anaesthesia was thereby avoided.

The effect of sodium cyanide on the respiration when depressed by a constant grade of intracranial pressure

In this series of experiments 21 dogs were used. The sodium cyanide was used in 0.01N solution. With large animals, 5 cc. of this solution may be required to give a marked stimulation of the respiration. In some cases, it is more convenient to use a 0.02N solution. In the first experiments single rather rapid injections were made. The cyanide acts very rapidly. The response always occurs within 8 to 15 seconds, depending somewhat upon the dose given. The duration of the stimulations varies greatly in different experiments. Thus in experiment 17, an intracranial pressure of 130 mm. paralyzed the respiration in three separate determinations. During cessation of the respiration on the third trial, a dose of 3 cc. 0.01N sodium cyanide was given. After a latent period of 12 seconds, the respirations returned and continued with perfect regularity and with the normal volume of tidal air for 1 hour, when the intracranial pressure was further increased to 142 mm. This again paralyzed the respiration, when, holding the same intracranial pressure, another dose of 2 cc. 0.01 N sodium cyanide was given. This again reinstated regular respirations which continued for 4 minutes when the

intracranial pressure was further increased to 174 mm.   When the respiration ceased, the blood pressure started falling and a vagal pulse developed.   Previous to the last increase of intracranial pressure, no significant changes occurred in the blood pressure.   At this time the blood pressure fell below the intracranial pressure so that the administration of cyanide was without avail because the cyanide did not reach the center and immediate decompression was necessary to save the animal.   In this experiment the cyanide stimulation following a single injection lasted a very unusually long period of time.   Later in this same experiment, the stimulation, following a single dose of sodium cyanide was of the usual brief duration, namely, only 55 seconds.   It is certain that the cyanide ordinarily is rapidly destroyed in the body and stimulation lasting an hour following a single injection must be due to remarkably slow destruction of the substance or the stimulation must last long after the cyanide has disappeared. The view that the cyanide is more slowly destroyed in these cases seems highly improbable because in the average animal the stimulations last only a minute and we would have to assume a rate of destruction of only one-sixtieth of the normal.   It would seem that in some cases the center has a certain inertia and when the administration of cyanide causes it to discharge at a certain tempo, that tempo tends to be maintained after the cyanide is gone.   This inertia seems to vary in the same animal (experiment 17) at different times due to factors which can not be analyzed at present.   We know that cyanide is rapidly destroyed in the body and converted into the sulpho-cyanide.   We shall later present data to show the rate of detoxification of sodium cyanide in the body.   It may be stated here, however, that, in most cases, the stimulation of the respiration only lasts as long as the cyanide remains as such in the body.   Often a series of injections of sodium cyanide cause a series of almost exactly similar responses.   A series of similar responses in the dog (experiment 18) with the respiration paralyzed by intracranial pressure is shown in figure 6.

Figure 7 shows a series of very similar responses of the respiration in man following repeated injections of 1 cc. 0.02x solutions.   The patient was unconscious.
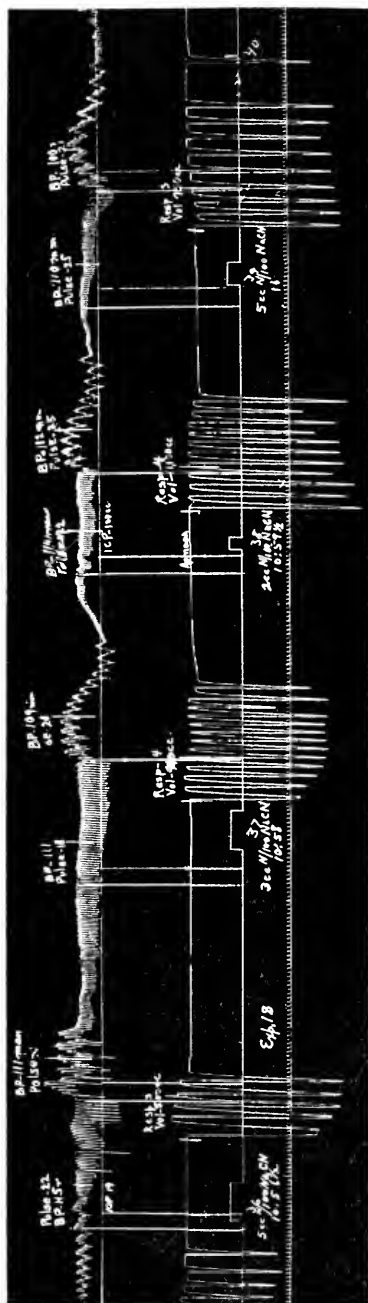
FIG. 6. Upper tracing, carotid blood pressure. Straight line just beneath it—intracranial pressure. Third tracing, respiratory record.
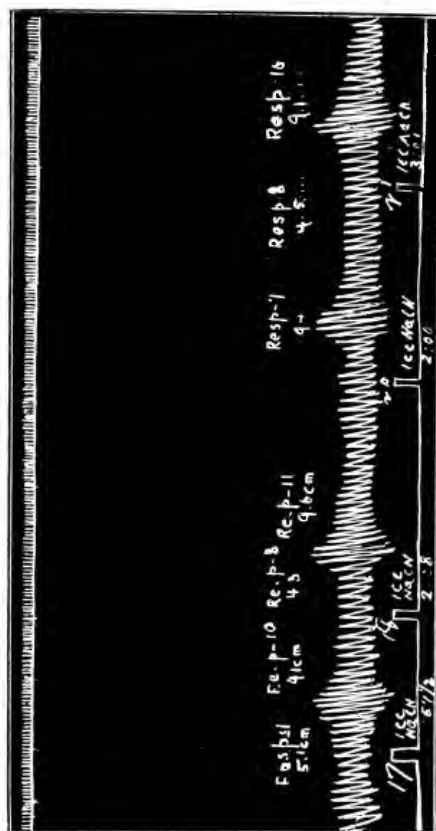
FIG. 7. RESPIRATORY TRACING IN MAN

Upper record, time in seconds. Second record, respiratory tracing. Third record, marking pen. Individual injections of 1 cc. of 0.02N sodium cyanide were given, as indicated by the marking pen.

Continuous sodium cyanide injections

Having often observed the series of stimulations which follow a series of single injections of sodium cyanide, it occurred to us that if the drug were given continuously these periods of stimulation might be made to coalesce and a continuous stimulation of the respiration obtained. The cyanide was, therefore, given at a slow rate from a burette by the gravity method in a series of experiments. The advantage of injecting the drug by the gravity method from a burette is that the rate of injection can be nicely controlled by the height to which the burette was raised or by the adjustment of a screw clamp on the rubber tubing between the burette and the needle. By this means it was found that instead of having a series of cyanide stimulations, the stimulation could be made continuous and the extent of the stimulation accurately controlled by the rate of injection. In the continuous injection of sodium cyanide, the respiration depends entirely upon the rate of injection. If the rate of injection is below a certain minimum neither stimulation nor depression will result no matter how long the injection is continued. The maximum rate at which the sodium cyanide can be injected for a considerable period of time without causing any response represents the maximum rate of detoxification of the substance in the body. The nature of this detoxification consists, in all probability, in the transformation of cyanide into sulphocyanate. As the rate of injection is increased the first effect noted is stimulation of the respiration. Usually when the respiration is depressed, this stimulation consists both of an increase in rate and an increase in amplitude of the respiration. As the injection is continued, the effect again depends on the rate of injection. If the rate of injection is not very far above the rate of destruction of the substance in the body, the stimulation will last almost indefinitely because under these conditions we maintain a concentration of sodium cyanide in the body which is the best level for stimulation, but, if the rate of injection is very considerably above the rate of destruction of the substance in the body, sodium cyanide will accumulate and within a vary-

ing time, depending on the rate of injection, the concentration of the sodium cyanide will reach a point which will depress the respiration. In our continuous injection work, it became the practice, in case it was necessary to produce immediate stimulation of the respiration, to inject 5 cc. 0.01N sodium cyanide very rapidly. When the effect was passing off the continuous injection of 0.5 cc. of 0.01N per minute was started. Finally the rate of injection was very carefully increased or decreased to a point which would produce the desired rate of stimulation. The proposition is quite similar to that noted in giving an anesthetic. It requires a higher concentration of anesthetic to induce anesthesia than it does to maintain it and the situation is quite the same in the stimulation of the respiration by sodium cyanide. If the stimulation tends to increase gradually at a given rate of injection, the rate of injection must be reduced. It occasionally happens, however, that the respiration does not respond well to sodium cyanide and, in this case, the drug must be discontinued.

We would now like to present the figures as to the relation of the response to the velocity of injection which we have found in our experiments. The rate of detoxification and the rate required for stimulation in different animals varies somewhat and has to be determined in each animal.[1]

Experiment AB. The weight of the animal was 7 kilos. The cyanide was injected at the rate of 3 cc. per minute for a period of 4.5 minutes, making a total injection of 14 cc. during the interval. This rate of injection during depression of the respiration by intracranial pressure caused a very marked initial stimulation of the respiration but the effect lasted only a very short time and the rate is obviously too fast for therapeutic purposes.

Experiment AD. The weight of the dog was 8 kilos. No intracranial pressure was applied in this experiment. An injection of 4 cc. 0.01N

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[1]Mr. Adolph M. Hanson, in conjunction with one of us (A. S. L.) has determined the maximum rate at which 0.01N sodium cyanide can be injected intravenously in dogs for a period of 20 minutes or longer without producing any symptoms whatever. This was found to be 0.4 cc. per minute. This we regard as the rate at which the average dog can completely detoxify the cyanide, namely, 0.2 mgm. per minute. It should be stated that this work was done on the decerebrate dog as well as the anaesthetized normal dog.

sodium cyanide over a period of 8 minutes caused definite stimulation of the respiration, the volume of ventilation increasing 17 per cent. The rate of injection was 0.5 cc. (0.25 mgm.) per minute. There was no change in the blood pressure or pulse rate. The rate of the respiration remained constant. This is approximately the threshold rate of injection for dogs.

An injection of 25 cc. in a period of 20 minutes , or 1.25 cc. per minute, caused a very great increase in the amplitude of the respiration, but with constantly decreasing rate until the respiration became paralyzed. This rate of injection is also obviously too rapid for this animal.

Experiment ADE. The animal weighed 8.8 kilos. 1.5 cc. per minute were given over a period of 16 minutes, with an increase in the ventilation of 35 per cent and an increase in the respiratory rate of 45 per cent. This was at the rate of 0.17 cc. per kilo, per minute. This injection was made with intracranial pressure at zero and at this time the respiration was good. The only additional point of interest in this experiment was that, with the respiration greatly depressed by an increase in the intracranial pressure, a rapid injection of 5 cc. sodium cyanide caused a great stimulation of the respiration, the rate being increased 230 per cent (18 to 60) and the ventilation 265 per cent (1500 cc. to 5500 cc.) of the normal. Sodium cyanide was then given at a slow rate, 11 cc. in 11 minutes or 1 cc. per minute, and the respiration continued good under the same intracranial pressure for 12 minutes— at which time the intracranial pressure was again raised and the respiration became embarrassed The rate of injection here used, namely, 1 cc. per minute was at the upper limit of therapeutic dosage.

Experiment AI. In this experiment we were dealing with an animal peculiarly susceptible to anemia of the respiratory center as shown by the fact that with the intracranial pressure at 110 mm., blood pressure being 130 mm., the respiration was paralyzed within a period of less than 2 minutes. Slow injection of sodium cyanide at the rate of 7.5 cc. in 4 minutes or approximately 2 cc. per minute re-established the respiration which again ceased, however, after about 3 minutes and decompression was necessary. 2 cc. per minute is decidedly above the therapeutic dosage for most dogs. Following decompression the respiration returned and remained good for about 24 minutes when it became greatly depressed without any further application of intracranial pressure. The circulation was in excellent condition at this time. The respiratory depression continued and became more severe until the respiratory rate was reduced to 5 respirations per minute, (the normal rate had been 72)

and the respirations were quite shallow the ventilation being only 240 cc. per minute (normal having been 2520 cc.). A rapid injection of 1 cc. sodium cyanide resulted within 20 seconds in an increase of respiratory rate to 24 per minute and a ventilation of 1362 cc. per minute. This stimulation passed off in about 30 seconds when the rate was 12 per minute and the ventilation 600 cc. per minute. A slow injection of sodium cyanide was then started and 6.3 cc. were given during a period of 8 minutes, the average rate being 0.8 cc. per minute. During this
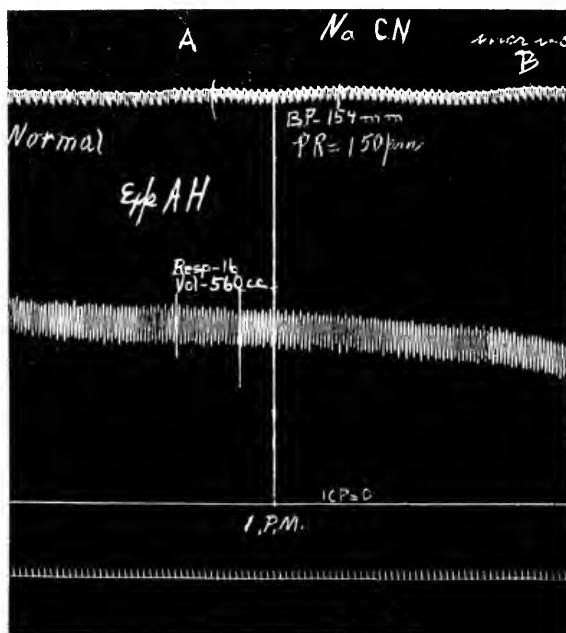
FIG. 8. Experiment AH. Upper record, carotid blood pressure. Next, respiratory record, base line and time in seconds. No intracranial pressure. See protocol.

injection the respiratory rate was 20 per minute and the ventilation 1500 cc. per minute. The injection was then stopped and the stimulation continued 40 seconds when the respiration again became paralyzed and vagal pulse developed. Later, when the pulse and respiration were again normal, increased intracranial pressure was again applied and the respiration began to be depressed when the intracranial pressure was 76 mm. below the blood pressure, again showing unusual sensitivity to medullary anemia. Paralysis of the respiration developed when the

intracranial pressure was 25 mm. below the blood pressure. The administration of sodium cyanide relieved the paralysis of the respiration. 5.5 cc. were given in 3.5 minutes, the rate being 1.6 cc. per minute. The respiration gradually become depressed so that decompression was necessary. 18.5 cc. of the cyanide was given continuously over a period of 18 minutes, when the respiration gradually ceased, the heart continuing to beat for five minutes.

Experiment AH. In this experiment, begun at 1.00 p.m., before the application of intracranial pressure or the giving of sodium cyanide, the dog had a blood pressure of 154 mm. and a pulse rate of 150. The respiratory rate was 96 and the ventilation 3360 cc. per minute (fig. 8). Intracranial pressure was gradually applied and at 1.07 it reached 115 mm. After maintaining this pressure for 1 minute the respiration became greatly depressed and decompression was done. This was repeated three times and each time there was marked depression or paralysis of the respiration. The fourth time that this intracranial pressure was applied the respiration ceased in $1\frac{1}{2}$ minutes and, at 1.20, the slow continuous injection of sodium cyanide started (fig 9.). This intracranial pressure was then maintained unaltered for 2 hours and 3 minutes without any further embarrassment of the respiration, during which time the sodium cyanide was continuously administered. The respiratory rate and ventilation at various times during this experiment follows:

TIME	RESPIRATION RATE PER MINUTE	VENTILATION	BLOOD PRESSURE	INTRACRANIAL PRESSURE	REMARKS.
		cc.			
1.00	96	3360	154		Normal (fig. 8)
1.20	3	375	124	114	Sodium cyanide injection started during depression (fig. 9)
1.28	84	4200	160	114	
1.53	54	6300	153	114	
3.20	54	7470	140	114	Fig. 10

At 3.23 after maintaining a rate of ventilation far above the normal with the intracranial pressure at a point which embarrased the respiration in the absence of sodium cyanide, the intracranial pressure was increased to 138 mm. while the administration of the sodium cyanide was continued. The blood pressure rose from 140 to 158 mm.; vagal pulse developed. The blood pressure then fell very gradually until 3.38, when it fell precipitously and death occurred at 3.41.
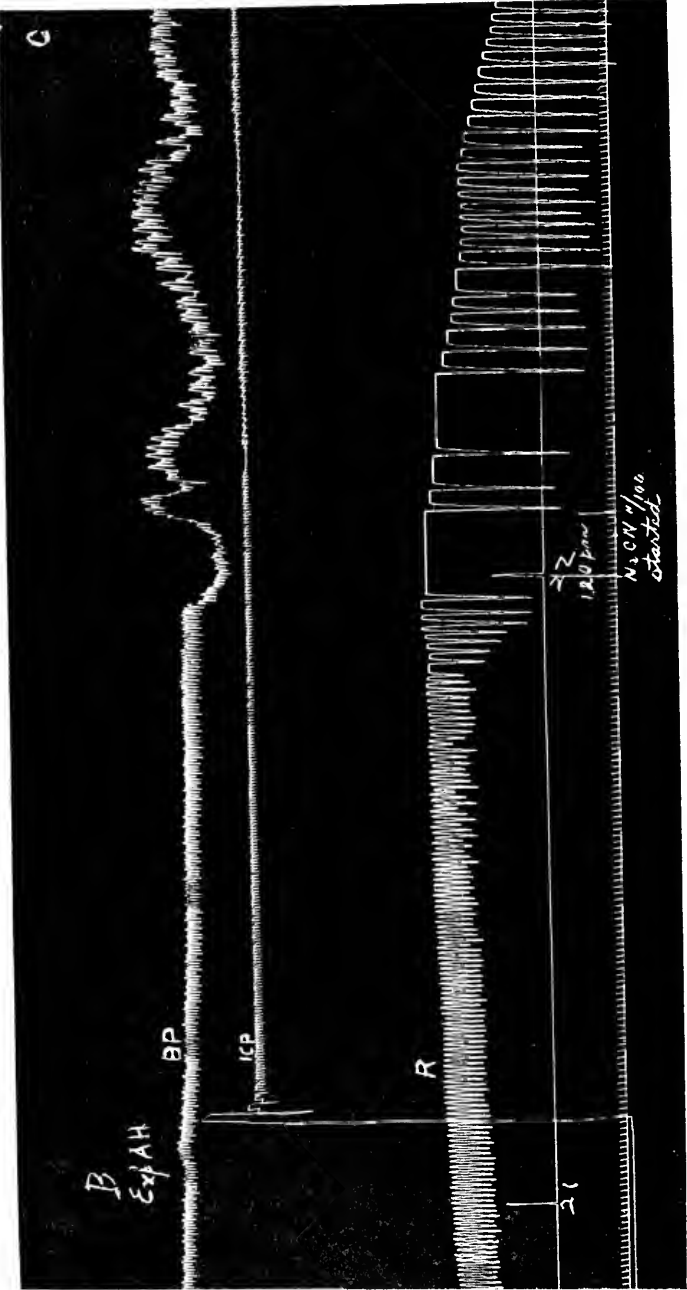
FIG. 9. The tracings are the same as in figure 8 except that the one below the carotid blood pressure is intracranial pressure. At 22, slow continuous injections of sodium cyanide started.

This experiment shows most strikingly the power of sodium cyanide to stimulate the respiratory center when depressed by increased intracranial pressure.

The conclusions to be drawn from the work on sodium cyanide under a condition of a constant grade of intracranial pressure are as follows:

1. The drug is the most reliable stimulant for the respiratory center known.
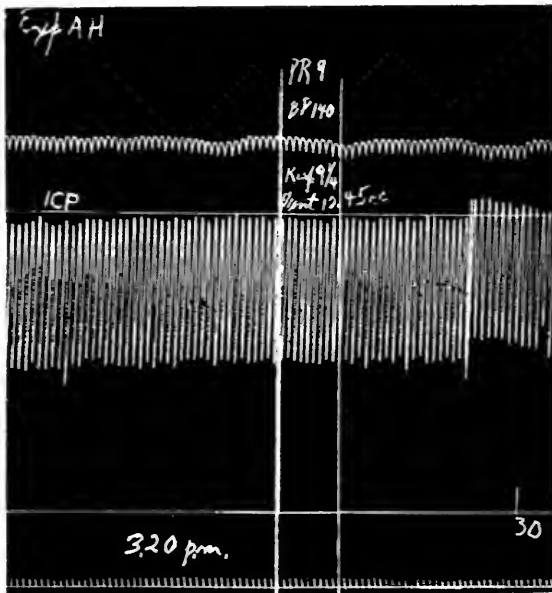
Fig. 10. The tracings are the same as in figure 9 after two hours run under increased intracranial pressure with slow continuous injections of sodium cyanide.

2. The duration of stimulation following single rapid injections is usually short, less than 1 minute, but occasionally the stimulation lasts as long as 30 minutes.

3. The stimulation may be made continuous by a slow continuous injection of the drug, and in this connection the following points should be noted:

a. The rate of injection for the best therapeutic effect varies somewhat in different animals.

b. If the rate of injection is too rapid the stimulation of the respiration passes off and depression supervenes. The best rate for therapeutic usage in the dog averages about 0.5 cc. of 0.01N sodium cyanide per minute to maintain a stimulation of the respiration which has been already initiated by a more rapid injection.

The effect of strychnine sulphate on the respiration when depressed by a constant grade of intracranial pressure

Eleven experiments were performed to determine the efficacy of strychnine. In the majority of cases, strychnine did not stimulate the respiration. In about 25 per cent of the cases distinct improvement in the respiration was noted following strychnine medication. The strychnine was given during respiratory depression, induced by increased intracranial pressure and the intracranial pressure was maintained during the action of the strychnine.

Stimulation of the respiration by strychnine was seen in experiment 21. The intracranial pressure was increased to 113 mm. with the mean blood pressure 122 mm. This caused stoppage of the respiration in 2 minutes. One minute after subjecting the animal to this increased intracranial pressure, the respiration became very much impaired and the rate very slow. One and one-quarter minutes after the intracranial pressure was applied, 0.37 mgm. strychnine sulphate were given intravenously. Following the injection the depression of the respiration continued for almost 2 minutes. One minute following the injection, there was a complete paralysis of the respiration lasting 30 seconds following which the stimulating action of the strychnine manifested itself and the respiration continued for 27 minutes. During this period the respiration was regular in rate and the ventilation was equal to the normal. The pulse was somewhat irregular for the first 8 minutes after which it became more normal and continued in excellent condition throughout the remainder of the 27 minutes. At the end of this period the intracranial pressure was further increased to 131 mm. The respiration soon became embarrassed and decompression was necessary. After a period of rest, an intracranial pressure of 113 mm. was again applied. Within 1 minute, this caused complete paralysis of the res-

piration for a period of 1 minute. At the beginning of the respiratory embarrassment, 0.25 mgm. of strychnine sulphate were injected. After 1 minute, the respiration returned and gradually increased so that the ventilation was excellent for a period of 8 minutes, when the respiration again became slow and irregular, forcing us to lower the intracranial pressure gradually to 92 mm. The animal was then decompressed, and, after a period of rest, the intracranial pressure was increased to 128 mm.; mean blood pressure was 128 mm. at this time. This caused embarrassment of the respiration within one minute, when 0.25 mgm. strychnine sulphate were given. The drug reëstablished the respirations which were regular. The rate was also good and the ventilation was above normal for the animal. After 7 m'nutes the respirations again became quite slow, but were regular and deep. Twenty minutes after giving the strychnine the respiration became extremely embarrassed, the blood pressure fell rather suddenly and the animal was decompressed 23 minutes after the strychnine was given. It is evident, therefore, from this experiment, that for 23 minutes the strychnine enabled the animal to endure a degree of intracranial pressure which, without the drug, would have been fatal in very much shorter time.

Experiment 23. This experiment also illustrates the stimulating action of the strychnine. In this experiment with an intracranial pressure of 103 mm. and a mean blood pressure of 106. mm., the respiration became paralyzed within 30 seconds. The injection of 0.12 mgm. strychnine sulphate resulted in a return of the respiration which lasted 8 minutes, when the respiration again became embarrassed and the animal was decompressed. Following the giving of the strychnine, the blood pressure rose gradually from 103 mm. to 112 mm. Here the strychnine terminated a period of respiratory paralysis, but the relief only lasted approximately 8 minutes. After a period of rest, the animal was again subjected to an intracranial pressure of 102 mm., which caused paralysis of the respiration within 30 seconds. 0.18 mgm. of strychnine sulphate were given. The drug caused no change in the blood pressure, but caused a resumption of the respiration, which, though slow, was regular and continued for 4.5 minutes, when the respiration again became paralyzed. At this time, an injection of 0.25 mgm. were given. Following this injection, the blood pressure fell, but in spite of the fall of blood pressure the respiration returned within 30 seconds and continued for 4 minutes with a constantly falling blood pressure.

In this experiment, therefore, strychnine in two instances interrupted paralysis of the respiration and caused its re-instatement. Stimulation of the respiration was apparently independent of blood pressure changes because it occurred in some cases with a rising blood pressure and in some cases with a falling blood pressure.

The longest period of stimulation of the respiration in this experiment was 8 minutes.

From the above experiments the following conclusions are reached:

1. The stimulating action of strychnine sulphate on the respiration was only apparent in 25 per cent of the animals which we studied.

2. In those animals in which strychnine produced a favorable response its beneficial effects were clean cut and outspoken.

3. The stimulating action of strychnine lasts for a much longer time following a single injection than in the case of sodium cyanide.

4. If strychnine fails to improve the condition of the animal by stimulation of the respiration, it does no harm to the animal in the therapeutic doses used.

The effect of atropine sulphate on the respiration when depressed by a constant grade of intracranial pressure

Seven experiments were performed, using this drug in doses varying from 0.05 to 1.0 mgm.

Experiment 15. 0.25 mgm. of atropine sulphate were given intravenously. This dosage removed the vagal pulse which was manifest at the time and thereby resulted in a sudden rise, followed by a rapid fall of blood pressure. Stimulation of the respiration lasted 2 minutes and death resulted within $2\frac{1}{2}$ minutes due to failure of the circulation. The failure of the circulation following atropine will be discussed later.

Experiment 18. Three doses were given, namely 0.05 mgm., 0.05 mgm. and 0.12 mgm. This paralyzed the vagal endings. At the time the drug was given, there was paralysis of the respiration. The medication resulted in re-instatement of the respiration for about three minutes, but the blood pressure fell continuously as it often does after atropine and necessitated decompression.

Experiment 25. The drug was given in dosage of 0.084 mgm. when the respiration was greatly depressed. This caused no stimulation of the respiration whatever. The vagal tone in this animal was practically zero and the giving of the atropine caused no increase in the pulse rate. The customary depression of the circulation which follows the administration of atropine in asphyxia failed to occur. Later, in this experiment, when the respiration was greatly depressed from the previous application of intracranial pressure, but with the intracranial pressure at zero at the time, another dose of 0.12 mgm. atropine was given without effect on the circulation or respiration. In this experiment, therefore, atropine totally failed to stimulate the respiration and did not have the deleterious effect on the circulation that has been noted so frequently by us in experiments where there existed a marked vagal tone, due to asphyxia at the time of the administration of atropine.

Experiment 26. With the blood pressure at 70 mm. and an intracranial pressure of 56 mm., the respiration was much embarrassed, when 0.062 mgm. atropine sulphate was given. The rate increased within 30 seconds from 15 to 21 per minute, but the ventilation remained the same. The stimulation of the respiratory rate only lasted about 1 minute, when the respiration became extremely shallow and decompression was necessary 2 minutes after the atropine was given. This dose of atropine paralyzed the vagal endings and the blood pressure gradually fell until decompression was done. Fourteen minutes later with the intracranial pressure at zero, the respiration was greatly depressed, and the blood pressure was 67 mm. An injection of 0.077 mgm. of atropine sulphate was given without effect on the circulation or respiration and four minutes later, 0.2 mgm also caused no effect on the circulation or respiration. In this experiment, therefore, we may say that atropine failed to manifest any stimulating action on the respiration.

Experiment 27. The respiration was paralyzed when the intracranial pressure was 100 mm. and the blood pressure was 125 mm. At this time, 0.062 mgm. atropine sulphate was administered. The animal possessed very little, if any, vagal tone at this time and no change in the pulse rate was noted. There was no stimulation of the respiration and decompression was necessary. This dose paralyzed the vagal endings, as proved by stimulation of the left vagus. On decompression, the respiration returned and was of excellent rate and depth. Later in the experiment, 0.074 mgm. atropine sulphate failed to stimulate the respiration when depressed by intracranial pressure. The same result was obtained on the injection of 0.125 mgm. and 0.250 mgm.

Experiment 28. The first dose of atropine sulphate (0.075 mgm.) was given when the respiration was not depressed. This had no effect on the respiration. A second dose of 0.15 mgm., given when the respiration was depressed by intracranial pressure, caused no stimulation of the respiration but on the contrary the depression of this function increased following the administration of the drug. A third dose of 1 mgm. of atropine sulphate failed to cause stimulation of the respiration and exercised no deleterious effect on the circulation.

Experiment 29. The first injection of 0.075 mgm. failed to stimulate the depressed respiration. A second dose of 0.15 mgm. was given during paralysis of the respiration, due to intracranial pressure. At the time of this injection, the intracranial pressure was 160 mm. and the blood pressure 100 mm. The drug paralyzed the vagal endings and caused the blood pressure to rise precipitously from 100 mm. to 188 mm., i.e., above the intracranial pressure. This increased blood supply to the respiratory center caused a return of the respiration which endured for 80 seconds. The blood pressure then fell precipitously as has been so often noted following atropine under these conditions.

From the foregoing we conclude:

1. Atropine, in certain animals, causes slight stimulation of the respiration.

2. This stimulation is never marked and is seen principally in those cases where there exists a marked vagal tone with the consequent low blood pressure. The removal of the vagal tone causes a marked rise of the blood pressure and a consequent increase of blood supply to the respiratory center. The increased blood supply to the center results in stimulation of the respiration, but the beneficial effects of this are lost by the fact that the blood pressure falls rapidly in spite of the increased ventilation and circulatory collapse results. It follows, from our work, that the removal of vagal inhibition from the partially asphyxiated heart has a very deleterious effect and results in the speedy death of the animal.

3. The use of atropine to stimulate the respiration in asphyxia is worse than useless. If there exists a marked vagal tone at the time of its administration, the death of the animal is hastened. If there does not exist a marked vagal tone at the time of its administration, a stimulating effect on the respiration of even brief duration is rarely seen.

The effect of caffeine citrate on the respiration when depressed by a constant grade of intracranial pressure

Three experiments were performed in which caffeine citrate was injected intravenously as a respiratory stimulant. The dose varied from 0.04 grams to 0.16 grams. In no case were we able to obtain clear cut indication that caffeine citrate stimulated the respiration, which had been depressed by increased intracranial pressure. After a certain quantity of caffeine citrate had been given in each case, marked depression of the circulation was noted, the blood pressure falling to shock level. The administration of sodium cyanide usually resulted in stimulation of the respiration after caffeine failed to stimulate.

Caffeine citrate is worthless as a respiratory stimulant when the respiration is depressed by increased intracranial pressure.

The effect of lactic acid on the respiration when depressed by a constant grade of intracranial pressure

Six experiments were performed with lactic acid. The dosage varied from 2 cc. 0.05n to 2 cc. 0.1n. In none of the experiments were we able to demonstrate a clear cut stimulation of the respiration by lactic acid and, after this drug failed to stimulate, sodium cyanide produced the usual stimulation.

Lactic acid is worthless as a respiratory stimulant when the respiration is depressed by increased intracranial pressure.

DISCUSSION OF RESULTS

We would like to emphasize the fact that we have studied the action of certain drugs, reputed to be respiratory stimulants on the respiration when it is depressed by increased intracranial pressure. We believe that the relative value of these drugs as stimulants for the respiration, when the respiration is depressed by other means than anemia of the medulla, cannot be inferred from our experiments. It is probable that our results will apply, however, in many cases of respiratory depression from medullary anemia produced by other means than increased intracranial pressure, such as hemorrhage and other disorders of the circulation.

We have found sodium cyanide to be the most reliable stimulant of the respiration under all conditions under which we have worked in connection with the increased intracranial pressure. We have found that it exercises a stimulating action on the respiratory center directly and independently of any change it may produce upon the circulation. In many cases, the injection of sodium cyanide causes some change in the blood pressure, but this is usually insignificant with therapeutic dosage. The clinical application of part of this work has already been published by Loevenhart, Lorenz, Martin and Malone (4).

The mechanism, by which the sodium cyanide causes stimulation of the respiration, is of great interest. It was pointed out by Gasser and Loevenhart (5) that the pharmacological action of cyanide on the medulla and cortex is exactly similar to the effects produced by any means of lessening oxidation in these centers (carbon monoxide, asphyxia, etc.)

In view of Geppart's (6) work and the complete similarity of symptoms between sodium cyanide (or hydrocyanic acid)and all other means of lessening oxidation within these centers Gasser and Loevenhart (5) were led to conclude that hydrocyanic acid acts entirely by depressing oxidation in the centers.

It was further pointed out by Loevenhart (7) that the response to reduced oxidation depends on three factors, namely, the suddenness with which oxidation is reduced in the centers, the extent to which oxidation is reduced, and the condition of the centers at the time of the reduction. If oxidation is reduced to too low a level, we obtain depression and not stimulation. The question, therefore, presents itself why hydrocyanic acid should be able to stimulate the respiratory center when it is already depressed by reduction of oxidation as a result of increased intracranial pressure. There is an apparent paradox here for we should rather expect that the hydrocyanic acid would further depress respiration under these conditions. We have no explanation to offer, but it is possible that the change in functional activity of the center depends principally upon the *change* in the rate of oxidation rather than upon the absolute rate. The important thing is the fact that cyanide does stimu-

late under these conditions. The explanation of its modus operandi must await further facts and experiments and we content ourselves at the time in simply pointing out that our experimental finding is not entirely in keeping with previously published views from this laboratory as to the mode of action of hydrocyanic acid and the relation between functional activity and oxidation.

SUMMARY AND CONCLUSIONS

Sodium cyanide is the most reliable stimulant to the respiration, when depressed by increased intracranial pressure, that we have studied. It exercises its stimulating action on the respiratory center directly and acts independently of any change which it produces in the circulation.

The stimulation of the respiration by sodium cyanide is certainly among the most striking, reliable and prompt responses of the animal mechanism to a drug.

The changes in the blood pressure following therapeutic doses of sodium cyanide are insignificant. The effects of sodium cyanide last but a very brief period, usually not over 1 minute, but occasionally stimulation may last as long as 30 minutes. Stimulation of the respiration following single injections of cyanide may be repeated at will. By giving cyanide continuously at the proper rate, continuous stimulation of the respiration may be maintained for hours.

Thus, with the intracranial pressure elevated to a point which embarrassed the respiration, it was possible by the continuous injection of sodium cyanide to maintain respiration at this level of intracranial pressure and the degree of ventilation was very accurately controlled by the rate at which the cyanide was administered.

Sodium cyanide must be administered intravenously. No other method of giving the drug is at all satisfactory. The dosage of sodium cyanide for stimulation of the respiration in the dog by single rapid injections is 1 to 3 mgm. The dosage for continuous injection to maintain an already established stimulation is approximately 0.25 mgm. (0.5 cc. 0.01N solution) per minute.

Strychnine sulphate

Strychnine sulphate, given intravenously, stimulated the respiration in about 25 to 50 per cent of our experiments. The stimulation of the respiration by strychnine is not so prompt nor reliable as in the case of sodium cyanide, but the stimulation following a single dose of strychnine lasts much longer than in the case of the cyanide. Thus, the duration of the stimulation often lasts from 20 to 60 minutes. In case strychnine fails to stimulate the respiration, the animal suffers no damage as a result of its administration.

Atropine sulphate

Atropine sulphate is a most unreliable respiratory stimulant. In certain animals it causes slight stimulation of the respiration, but in most animals no stimulation is seen following its administration. Stimulation, when noted, is never marked and is seen principally in those cases where there exists a marked vagal tone and a consequent low blood pressure. The removal of the vagal tone by the atropine causes a marked increase in the pulse rate and a rise of blood pressure. The rise in blood pressure has a beneficial effect on the respiration but only for a very brief period of time because in our experiments the circulation collapsed with striking suddenness in spite of the transitory improvement of the circulation and respiration. It seems to us, from our experiments, that the relief of the vagal pulse by atropine, when the heart is in a condition of partial asphyxia, has a very deleterious effect on the heart. We, therefore, attribute the deleterious effect of atropine in our work to its action in relieving the vagal pulse, and believe this to be a great disadvantage to the already partly asphyxiated heart. It is our opinion, therefore, that the use of atropine in this condition is worse than useless.

Caffeine citrate

In some cases, brief but definite stimulation of the respiration by caffeine citrate was noted. In spite of this stimulation, its effect seemed to be harmful rather than beneficial, the harmful

effect probably being attributable to changes in the circulation although the cause of the deleterious action on the circulation was not investigated.

Lactic acid

Lactic acid is rarely an efficient stimulus of the respiration when depressed by increased intracranial pressure. In most cases, no stimulating action was observed.

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OBSERVATIONS UPON THE RESISTANCE OF THE RAT
TO CONSECUTIVE INJECTIONS OF STRYCHNINE

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The experiments herein reported were performed in connection with the investigation on the toxicity of strychnine to the rat (3), Mus norvegicus, for the purpose of obtaining some information as to the ability of this animal to dispose of (eliminate, fix and destroy) this drug. The data are of interest in that in this respect the rat was found to possess a very remarkable resistance. To a great extent this will account for the necessity for administering large doses of strychnine orally (20 to 25 mgm. of the alkaloid per kilo of rat) in order to secure a high percentage of fatalities.

Previous investigations on other animals. The ability of the cat, dog and guinea-pig to dispose of this drug has been studied by Hatcher and Eggleston (2). They showed that only a small percentage of the adminstered strychnine appeared in the urine, that it could be completely absorbed from the gastro-intestinal tract, and that the chief mechanism of protection lay in the ability of the animal to destroy it within the tissues. The respective species resistance in terms of minimum lethal subcutaneous dose per hour was found to be as follows: Cat, 12 to 25 per cent per hour; dog, 5 to 17 per cent; and guinea-pig, 18 to 57 per cent. These hourly coefficients represent the arithmetical mean obtained by dividing the time in hours between the first and last injections into the total amount of strychnine administered in excess of a single minimum lethal dose.

Plan of experiments. The experiments herein reported were planned so that some idea could be obtained of the resistance of the rat as judged by the relation of survivals to fatalities in a

given series, as well as by the coefficients determined arithmetically for each individual animal. The purpose of the first type of evidence was to serve as a check, since the second method tended to give low results unless the strychninization had been continuously kept up almost to the possible maximum. Accordingly a large number of rats were consecutively injected with the minimum lethal subcutaneous dose of strychnine sulphate (3 mgm. per kilo in 0.1 per cent concentration). The survivors were tested at two-hour intervals with fractional multiples of this dose. The experiments were performed practically simultaneously and the same syringe, solutions, technique, etc., were used. There exists, therefore, perhaps a better basis for comparisons than might be obtained from experiments performed on different days.

The results of a number of experiments in which strychnine was administered orally were also briefly reported. All the administrations were made by stomach tube (urethral catheter) and a record syringe to lightly etherized rats. The strychnine was in the following forms: The sulphate in solution; the alkaloid in 100 fine powder suspended uniformly in starch paste; and the sulphate dissolved in an aqueous solution of the food color amaranth.

No experiments to show that the strychnine was actually entirely absorbed from the gastro-intestinal tract were directly performed. Indirect evidence bearing upon this point will be presented. As no metabolism elimination or perfusion experiments were made, only the disposal will be considered. Theoretically this includes the storing or fixation, the elimination and the metabolism.

EXPERIMENTAL

Subcutaneous injection. The minimum lethal subcutaneous dose (3 mgm. strychnine sulphate per kilo in 0.1 per cent concentration) was injected into 31 well-nourished white rats, all of which weighed over 170 grams. Fifteen rats, or approximately 50 per cent, died. The deaths were distributed fairly evenly over the last three-quarters of the first hour and evidence of fairly rapid absorption was general.

At the beginning of the third hour the 16 surviving rats were divided into three groups and injected as follows:

Group 1 (five rats). Each received one-third of the minimum lethal dose, or 1 mgm. of strychnine sulphate per kilo. All survived, but were occasionally spastic.

Group 2 (seven rats). Each received one-half the minimum lethal dose, or 1.5 mgm. strychnine sulphate per kilo. Three died and four survived.

Group 3 (four rats). Each received two-thirds of the minimum lethal dose, or 2 mgm. strychnine sulphate per kilo. Three succumbed and one lived.

The animals in groups 1 and 2 which survived the experiments were given at the beginning of the fifth hour the following amounts:

Group 1 (original five rats). Each received 50 per cent of the minimum lethal dose, or 1.5 mgm. strychnine sulphate per kilo. All survived.

Group 2 (four out of the original seven rats). Each received 50 per cent of the minimum lethal dose, or 1.5 mgm. strychnine sulphate per kilo. All survived.

Great precaution, therefore, was exercised in injecting the animals of Group 1 at the beginning of the sixth hour as follows:

Group 1 (original five rats). Two received one-third of the minimum lethal dose, or 1 mgm. per kilo. Both died. Three received one-half of the minimum lethal dose, or 1.5 mgm. per kilo. Two died.

Oral administration. In experiments to be reported elsewhere (3) in detail it will be shown that a few rats survived an oral dose of 20 mgm. strychnine sulphate per kilo. These rats, however, at the end of the seventeenth hour gave no evidence of being strychninized.

In another series which was given larger doses of strychnine sulphate in combination with diatomaceous earth, strychninization was observed after the seventeenth hour.

Another series was given a solution of strychnine sulphate and the food color amaranth. If properly prepared such a mixture gives a clear solution, presumably of a combination of amaranth

with strychnine. Post mortem examination of the rats succumbing revealed that in some of them dye was present only in the cardiac end of the stomach. The pyloris was entirely blocked off by large masses of semi-dry uncolored stomach contents. Some, however, showed a small amount of dye in the duodenum, but such cases need not enter into this discussion.

It was also observed that the fatalities from strychnine by stomach tube (either the alkaloid in starch paste suspension or the sulphate in solution) usually occurred within two hours, while with the strychnine sulphate diatomaceous earth combination death was more delayed.

INTERPRETATION OF EXPERIMENTS

The interpretation of experiments of this nature is met by two difficulties, first, the unreliability of death as an experimental criterion, and, second, effect of fatigue, depression, etc., upon the subsequent toxicity of strychnine. The first of these objections may be dismissed by using enough animals to rule out extreme variations or anomalous results. The second objection may be neglected so far as the purpose of this report is concerned, since the evidence points to an underestimation rather than to an overestimation of the disposal. These data are, therefore, on the side of conservatism.

The results of the injections at the beginning of the third hour, as judged by the fatalities and survivals, show that approximately half of the rats will dispose of 50 per cent of subcutaneous minimum lethal dose in two hours, that all presumably will dispose of 33 per cent, and that occasionally one will dispose of 67 per cent. The propriety of the halving of these two hour coefficients in order to obtain the hourly disposal will be discussed later.

The explanation of the survival by all rats in group 1, of the injection at the beginning of the fifth hour (third injection), appears simple. These rats did not receive sufficient additional strychnine, which together with that still within the system, would equal a lethal amount. The behavior of group 2, however, is not

so easily explained, because it was to have been expected that one or two might have succumbed. Increased resistance, unusual power of disposal, survival by chance and fatigue, exhaustion or secondary depression from the strychnine were thought of at the time as being accountable for this. It should be mentioned that after receiving injections of strychnine the rats became sleepy or depressed, although they still showed hyper-excitability when stimulated. Because of the uncertainty as to the cause of the survival of the rats of group 2, the other (group 1) was again injected at the beginning of the sixth hour. Group 2 was not injected because it was anticipated at the time that some of them might have died during the next (sixth) hour.

These experiments on group 1 (during the sixth hour) show that the ability of these rats to dispose of strychnine was not greater than it was for other rats at the beginning of the third hour. Unfortunately these injections were planned for the purpose of testing for an increased resistance, whereas it developed that it would have been better to have planned on the constancy of the toxicity of strychnine. These tests on group 1 show that two rats were unable to withstand within one hour a dose of half of the amount that other rats were usually unable to withstand within two hours (compare group 3 second injection). There is, therefore, good reason for two other rats succumbing to a larger dose of strychnine. The fifth rat of this group, however, showed a high tolerance, and presumably represents an occasional exceptional instance. The survival of all of group 2 (fifth hour), therefore, probably was by chance.

The calculated amounts of strychnine disposed of by the rats are given in table 1, in terms of percentage of a minimum lethal dose per hour. A fatality was taken to indicate that the amount administered in the course of the experiment exceeded the capacity of the rat and the results are accordingly recorded as "less than — per cent," while a survival is recorded as "as much as or greater than — per cent." Data for some of the rats are entered in both columns because the experiments were continued long enough and also have a fatal termination. These data agree fairly well with the strictly experimental method of estimating the disposal.

The experiments dealing with the oral administration of strychnine have been cited because of their bearing upon the question of absorption from the gastro-intestinal tract. Those rats which escaped death evidently did so because the active strychnine present at one time in the body did not exceed an amount equal to the subcutaneous lethal dose for the individual. The accumulation of a lethal amount depended upon the rate of absorption exceeding the rate of disposal. An estimate of the balance displayed between these two opposing forces is

TABLE 1

Disposal of strychnine administered subcutaneously; arithmetical method of calculation

GROUP	NUMBER OF RATS	HOURS BETWEEN FIRST AND LAST INJECTIONS	DOSE IN EXCESS OF MINIMUM LETHAL DOSE	DISPOSAL IN TERMS OF MINIMUM LETHAL DOSE PER HOUR	
				Less than*	As much as or greater than†
			mg. per kilo	*per cent*	*per cent*
1	2‡	⎰4	2.5		20.8
		⎱5	3.5	23.3	
2	2‡	⎰4	2.5		20.8
		⎱5	4.0	26.6	
1	1	5	4.0		26.6
2	3	2	1.5	25.0	
2	4	4	3.0		25.0
3	3	2	2.0	33.3	
3	1	2	2.0		33.3

* Based on fatalities.

† Based on survivals.

‡ Two factors for same rats, but for different length periods and for different end result, e.g., fatality or survival.

obtained from the following observations. 1. Death from orally administered strychnine usually occurred within two hours. 2. Death was certain only from large doses such as 25 to 30 mgm. of the sulphate per kilo. The reason for administering arge doses, was obviously to increase the rate of absorption. A rat, therefore, had to be overwhelmed or it might survive.

Qualitative evidence bearing upon the ability of the stomach to absorb strychnine was obtained by administering dye with the strychnine. Since the dose administered was many times the

subcutaneous minimum lethal dose, and since the rat is a great deal more resistant to strychnine than some of the higher animals, these data do not relate to the general question of absorption of fatal doses in such animals as man, cats and dogs. They do show, that under the conditions tried, and the practical conditions necessary to poison rats, that a fatal dose of strychnine can be absorbed from the stomach of the rat.

The observation that strychninization occurs rather soon also speaks for rapid absorption whether it be from the stomach or intestine. The absence of signs of strychninization after the seventeenth hour in rats occasionally surviving very large doses of strychnine (20 mgm. or more of the sulphate per kilo) shows that presumably most of the strychnine had been disposed of by the rat. If any were unabsorbed it must have practically all passed out in the feces. That this probably does not occur is shown by the fact that rats receiving large doses of strychnine sulphate in combination with diatomaceous earth give signs of strychninization after the seventeenth hour.

Although no tests were made to prove that all the strychnine was absorbed from the gastro-intestinal tract, it is believed that the indirect evidence obtained is in complete harmony with the results of the direct experiments of Hatcher and Eggleston (2) on other animals. It is fair, therefore, to assume that in the rat strychnine is practically completely absorbed from the gastrointestinal tract.

CORRELATION OF THE EXPERIMENTS OR THE SIGNIFICANCE AND APPLICATION OF THE COEFFICIENT OF DISPOSAL OF STRYCHNINE

In the correlation of the experiments herein discussed it is necessary to bear in mind several facts. First that the intermittent subcutaneous injections of strychnine give at best (particularly with two-hour intervals) a discontinuous or uneven strychninization, whereas absorption from the gastro-intestinal tract can be more even and less interrupted because the source of supply of the drug is relatively large. Second, that strychnini-

zation seems to have disappeared by the eighteenth hour when large doses of strychnine are administered orally.

As judged by the relation of survivals to fatalities, 50 per cent of the rats could dispose of 50 per cent of a minimum lethal subcutaneous dose in two hours. This was corroborated by the arithmetically determined coefficients. On this basis it would take a rat eighteen hours to dispose of $4\frac{1}{2}$ minimum lethal subcutaneous doses, or 13.5 mgm. per kilo. Adding to this another dose, because the rapid disposal is dependent upon the presence of an appreciable amount of strychnine in the tissues, brings the total absorbed strychnine up to 16.5 mgm. per kilo. Some rats receiving 20 mgm. per kilo, however, have shown no signs of strychninization during the eighteenth hour. (It is not known how much sooner the strychninization disappeared.) Some explanation must be found therefore for the discrepancy between this calculation and this observation.

A possible cause might be found in the effect of the disposal upon the amount of strychnine present and this in turn upon the amount disposed of. Under conditions prevailing in the gastrointestinal tract the strychnine disposed of by the organism can be replenished because the supply is relatively large, whereas the absorption from the subcutaneous tissue is probably much more rapid and the source of supply is rapidly exhausted. The data determined by the subcutaneous method should then be reinterpreted on a relative basis, namely, as to the effect of the disposal upon the absolute amount present and as to the effect of this decrease on the absolute disposal during the next period. Such a point of view is tenable, because strychnine has been found in the urine of animals (1) (2) for several days after its subcutaneous administration, whereas on an absolute basis of disposal all strychnine should have disappeared in a few hours.

If the absolute amount of strychnine disposed of is a certain constant percentage of the amount present (which is constantly decreasing), the absolute amount disposed of for any given initial fractional part of a known period may be calculated as follows:

$\frac{1}{2}$ is eliminated in 2 hours

x is eliminated in 1 hour

$\frac{1}{2}$ equals $x + x (1 - x)$

x^2 equals $2x - \frac{1}{2}$

x equals $1 - \frac{1}{2} \sqrt{2}$

. x equals $1 - (\frac{1}{2})^{\frac{1}{2}}$ equals 29.3 per cent

y is eliminated in first 15 minutes ($\frac{1}{8}$ of two hours)

y equals $1 - (\frac{1}{2})^{\frac{1}{8}}$ equals 8.37 per cent

At the calculated elimination for the first fifteen minutes, if the strychnine were absorbed as fast as it was disposed of and there were present continuously a minimum lethal dose, there would be disposed of in one hour approximately one third of a minimum lethal subcutaneous dose (1 mgm. per kilo) or an entire dose in three hours. In seventeen hours a rat should have disposed of 17 mgm. per kilo, which together with approximately 3 mgm. within its system (so that it would still be strychninized at the beginning of the eighteenth hour) would bring the total amount to 20 mgm.

While this calculation may not entirely account for the amount of strychnine some rats are able to withstand in a given time, it accounts for the disappearance of more strychnine, and, therefore, agrees more closely with the observations in the oral experiments than the uninterpreted ratio.

These data are probably also of interest in connection with habituation of strychnine (1). Although acquired tolerance or habituation to drugs may be due to one or all of several distinct factors, it would seem significant that animals are able to dispose of this drug at such a rapid rate, and that any further increase in power developed to dispose of strychnine would not be considered as very probable.

CONCLUSIONS

The tolerance of the rat to repeated subcutaneous injections of strychnine has been studied. Evidence has been adduced to show that the coefficients of disposal which are necessarily expressed in terms of percentage of minimum lethal dose per

given period of two hours are too low. They should be rein-
terpreted to fit the case of oral administration in which large
amounts are administered at once and in which type of experi-
mentation the absorption of strychnine has an opportunity to
keep pace with the disposal. Accordingly, the coefficient of
disposal has been regarded as constant and the absolute amount
disposed of as a variable depending on the amount present. On
this basis the disposal of strychnine by the rat may reach at least
1 mgm. per kilo per hour. This occurs only when the strych-
ninization is kept constantly very close to the maximum limit,
by absorption from the gastro-intestinal tract.

The coefficients as determined arithmetically have been
checked by a new experimental criterion, namely, the relation of
survivals to fatalities in a given uniformly treated series and
comparison of one such series with another.

The extremely high tolerance of the rat (as well as of other
animals) to consecutive injections of strychnine would seem to be
significant in respect to the possibility of correlating this with the
failure to demonstrate as yet an habituation to this drug.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

VIII. MORPHINE

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In connection with our study of the hyperthermia (and hyperglycemia) produced by morphine in cats, which is the subject of another paper (1), we made some experiments on the influence of morphine upon the rate of ouput of epinephrin from the adrenals. With so widespread a nervous excitation as is seen in these animals after the administration of this drug, including the excitation of many sympathetic fibers, such as those which cause dilatation of the pupil and erection of the hairs, and the increased reflex excitability, it seemed not unlikely that the nervous mechanism governing the epinephrin output might also be excited. Not that there was the slightest reason to suppose that the symptoms could be due in any important degree to an increased discharge of epinephrin, since, as previously shown by us (2), all these symptoms are equally well seen in cats whose epinephrin output has been abolished or greatly reduced by extirpation of one adrenal and section of the nerves of the other. This is also the case when one adrenal and the major portion of the other have been removed, and the nerves going to the remaining fragment severed. The hyperthermia, which is so constant and conspicuous an effect of morphine in the cat, is also uninfluenced by this operation in respect either of its course and duration or of the maximum rise of temperature (as much as 4°C.). Nevertheless, as an item in the pharmacology of morphine, the question whether it affects the epinephrin output has a certain interest. We have been able to show that in the cat morphine

59

does cause a perfectly definite and considerable increase in the rate of output, which lasts for some time under our experimental conditions, although possibly more transient than the increase caused by strychnine,   On the other hand in the dog, at any rate with such doses as we have employed, there is either no change at all in the rate of output, or it may appear to be slightly increased. This agrees with our previous experience that in dogs anesthetised with morphine (administered some time before the experiment) and then with ether, there was no evidence of any increase on the ordinary average output.   The difference in the action of morphine upon the cat and dog is therefore as striking in this particular as in its other effects.

No attempt was made to determine the smallest dose which can produce a distinct increase in the cat.   From the considerable increases observed with the smallest doses employed, it would seem probable that some effect would be obtained with materially smaller amounts.   The cannula for collecting the adrenal blood was inserted into the inferior vena cava in the usual way, the abdominal aorta, but not the mesenteric artery or the coeliac axis, being tied.   The operation was performed under ether.   After administration of morphine, ether was either given in small amount or discontinued if the anesthesia was sufficient. Since the posterior end of the body is excluded from the circulation, the doses calculated on the weight of the portions of the body to which the drug had access would be greater than those given, which are calculated on the entire body weight (free from 'intestinal contents and urine).   The samples of adrenal vein blood were defibrinated, at once weighed and placed upon ice.   The assay (on rabbit intestine segments) was begun as soon as practicable after completion of collection of the samples, usually within an hour to an hour and a half.   The specimens collected after administration of morphine were, of course, assayed with an indifferent blood containing morphine.   Control tests showed, however, that even considerably greater concentrations of morphine than could possibly have been present in any of the blood specimens produced no noticeable effect upon the segments. In many instances the segment was atropinised after a series of

observations had been made, and the assays repeated on the atropinised segment. Approximately the same concentration was always obtained, but usually atropin increases the sensitiveness of the segment for epinephrin, and thus the assay can sometimes be made sharper. Sometimes the segment was atropinised at or near the beginning of the observations, especially if it was not responding with sufficient constancy to one and the same dose of adrenalin. As previously mentioned (3), a segment which seems rather unsatisfactory for the assay at first is often improved by atropin.

A typical protocol will now be given of an experiment with subcutaneous, and another of an experiment with intravenous injection of morphine.

Condensed protocol.   Cat 619; male; weight 3.39 kgm.

Anesthetised with ether.   Obtained indifferent blood from jugular vein.

9.55 a.m.   Cava pocket completed and cannula inserted. Pupils one-third to one-half maximum dilatation.

9.56½ a.m.   First specimen. 4.3 grams in 30 seconds (8.6 grams per minute).

9.57 a.m.   Second specimen, 10.0 grams in 90 seconds (6.7 grams per minute).

10.06 a.m.   Subcutaneous injection of 25 mgm. morphine sulphate. Blood pressure 125 mm. of mercury. No ether was necessary after the morphine injection. The eye reflexes were present, but the animal was quiet.

10.08 a.m.   Blood pressure 112; pupils half to two-thirds maximum.

10.21½ a.m.   Third specimen, 4.45 grams in 30 seconds (8.9 grams per minute).

10.22 a.m.   Fourth specimen, 12.1 grams in 90 seconds (8.1 grams per minute). Blood pressure 120. No change in the condition of the animal. No increase of reflexes; pads of feet dry; pupils unaltered since last observation.

10.55 a.m.   Blood pressure 100. It has been gradually falling. No change in symptoms.

11.05½ a.m.   Fifth specimen, 2.65 grams in 30 seconds (5.3 grams per minute).

11.06 a.m. Sixth specimen, 6.65 grams in 90 seconds (4.4 grams per minute).   Blood pressure 70.   The animal was quiet; no change in symptoms.

11.45 a.m. Some increase of reflex excitability.   Otherwise no change.

11.50½ a.m. Seventh specimen, 1.2 grams in 30 seconds (2.4 grams per minute).

11.51 a.m. Eighth specimen, 5.9 grams in 3 minutes (2 grams per minute).   Blood pressure 40.   Obtained indifferent blood.   Weight of the two adrenals 0.506 gram.

The second specimen (taken before injection of morphine) was shown to be stronger than 1:12,500,000 adrenalin, a little stronger than 1:7,500,000, weaker than 1:6,250,000.   The segment was then atropinised.   The second specimen was now found to be stronger than 1:10,000,000, somewhat stronger than 1:7,500,000, weaker than 1:6,250,000 (fig. 1, observations *16, 18* and *24*, confirmed by other observations).   The concentration is relatively small because of the large blood flow at the time this specimen was collected.   It was taken at 1:7,000,000, corresponding to an output of 0.0096 mgm. per minute for the cat, or 0.00028 mgm. epinephrin per kilogram per minute.

The fourth specimen, collected 16 minutes after injection of 7.3 mgm. of morphine sulphate per kilogram of body weight, was stronger than the second, but much weaker than the sixth (fig. 2, observations *46* and *52*) and than the eighth specimen.   It was stronger than 1:6,250,000, weaker than 1:3,750,000, somewhat stronger than 1:5,000,000(confirmed in each case by two sets of observations; tracings not reproduced).   It was assayed at 1:5,700,000, corresponding to an output of 0.0014 mgm. per minute for the cat, or 0.0004 mgm. per kilogram per minute. At this time the output had not increased much.

The sixth specimen, collected one hour after injection of morphine, was somewhat stronger than the eighth, and both were much stronger than 1:1,250,000 (fig. 1, observations *28* to *32*). The sixth specimen diluted with an equal volume of indifferent blood, to facilitate the assay, was weaker than 1:1,250,000, i.e., the sixth was weaker than 1:625,000, but decidedly stronger than 1:950,000 (fig. 2, observations *38* to *44*).   It was confirmed

that the sixth and eighth specimens were both weaker than
1:625,000 (fig. 2, observations *38*, *46* and *50*). The sixth speci-
men was taken at 1:750,000, corresponding to 0.0057 mgm. of
epinephrin per minute for the cat, or 0.0017 mgm. per kilogram
per minute (6 times the initial output). The eighth specimen
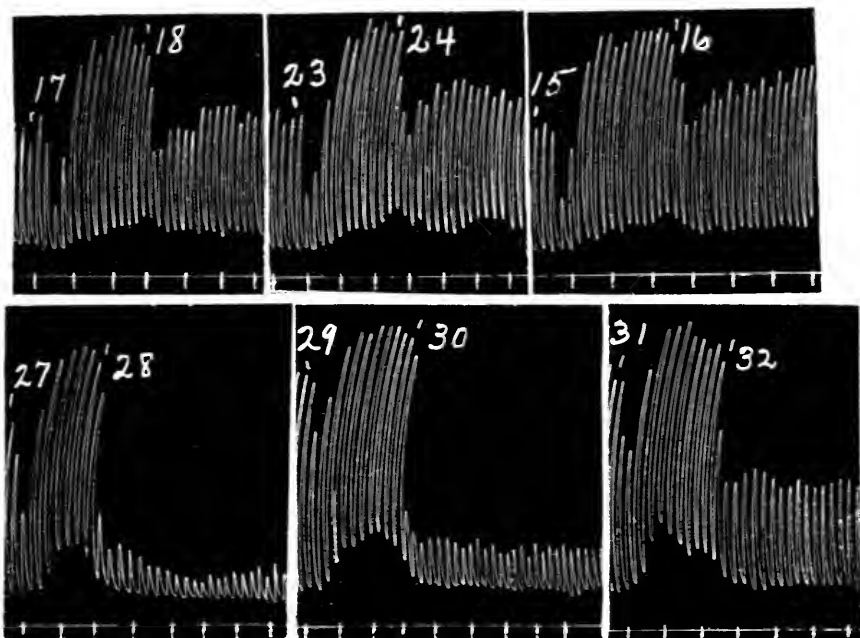
FIG. 1. INTESTINE TRACINGS; BLOODS FROM CAT 619

At *15*, *17* and *23* Ringer was replaced by indifferent (jugular) blood (obtained
before administration of morphine), and this at *16* by indifferent blood to which
was added adrenalin to make a concentration of 1:7,500,000, at *18* by indifferent
blood to which was added adrenalin to make a concentration of 1:6,250.000 and
at *24* by the second adrenal blood specimen (collected before administration of
morphine). At *27*. *29* and *31* Ringer was replaced by indifferent (arterial) blood
(collected after administration of morphine) and this at *28* by the sixth adrenal
blood specimen (collected one hour after administration of morphine), at *30*
by the eighth adrenal blood specimen (collected one and three-quarters hours
after administration of morphine), and at *32* by indifferent blood to which was
added adrenalin to make a concentration of 1:1,250,000. All the bloods were
diluted with three volumes Ringer (the adrenalin bloods after adding the adre-
nalin). As in all the figures, time tracing is given in half minutes. (Reduced
to two-thirds.)

was taken at 1:800,000, corresponding to 0.0025 mgm. per minute for the cat, or 0.0007 mgm. per kilogram per minute ($2\frac{1}{2}$ times the initial output). At the time of collection of the eighth specimen, (an hour and three quarters after injection of
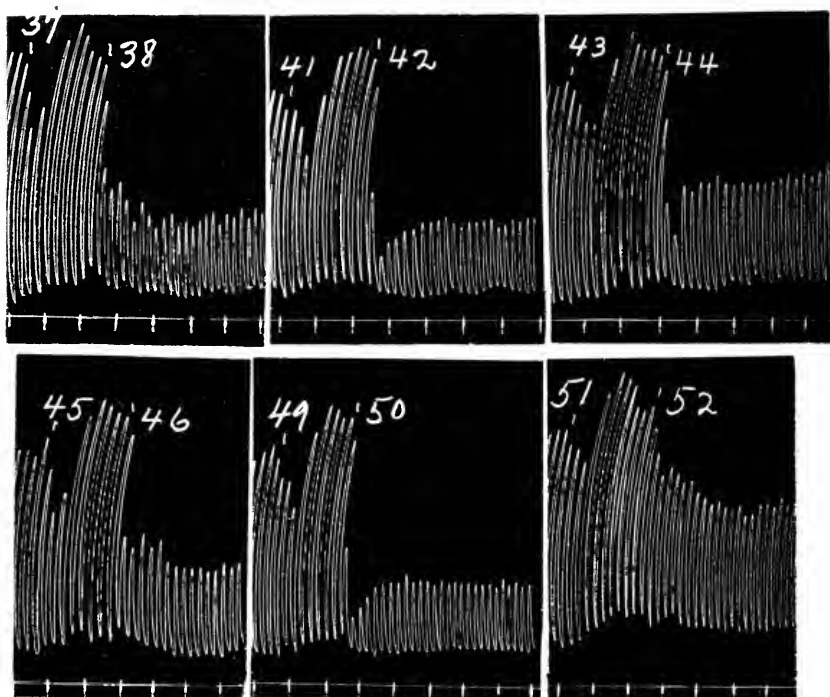
FIG. 2. INTESTINE TRACINGS; BLOODS FROM CAT 619

At *37, 41, 43, 45, 49* and *51* Ringer was replaced by indifferent blood (collected after administration of morphine) and this at *38* by the sixth adrenal blood specimen (collected one hour after administration of morphine) diluted with one volume of indifferent blood, at *42* by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000, at *44* by indifferent blood to which was added adrenalin to make a concentration of 1:1,900,000, at *46* by the eighth adrenal blood specimen (collected one and three-quarters hour after administration of morphine) diluted with one volume indifferent blood, at *50* by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000 and at *52* by the fourth adrenal blood specimen (collected sixteen minutes after administration of morphine). All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin, and the sixth and eighth adrenal blood specimens after adding the indifferent blood). (Reduced to two-thirds.)

the drug), the rate of output had again declined. It must be remembered, however, that the concentration in this specimen was high, and with the relatively small blood flow the full increase could not be shown in the calculated output if the limit of concentration in this animal had already been reached. But there is some evidence that morphine can drive up the concentration beyond the maximum ordinarily seen.

Condensed protocol. Cat 626; female; weight 2.16 kgm.

Anesthetised with ether. Obtained indifferent blood from abdominal aorta. Cannulas inserted and pocket completed at 9.00 a.m.

9.03½ a.m.   First adrenal specimen. 1.3 grams in 30 seconds (2.6 grams per minute)

9.04 a.m.   Second specimen, 5.13 grams in 2 minutes (2.56 grams per minute).

9.16 a.m.   Completed injection of 22 mgm. morphine sulphate nto jugular vein. B'ood pressure fell rapidly from 134 to 76 mm. of mercury, with slowing of heart. Started art ficial respiration and continued it during collection of third and fourth specimens. Spontaneous respiration was present, but was shallow at first.

9.16½ a.m.   Third specimen, 0.8 gram in 30 seconds (1.6 grams per minute).

9.17 a.m.   Fourth specimen, 4.75 grams in 3 minutes (1.6 grams per minute). Blood pressure 74. Spontaneous respiration now good. Pupils maximally dilated; eye reflexes present. Cat quiet, no ether required.

9.30 a.m.   Blood pressure 94. Condition of animal unchanged.

9.39½ a.m.   Fifth specimen, 1.3 grams in 30 seconds (2.6 grams per minute).

9.40 a.m.   Sixth specimen, 4.55 grams in 2 minutes (2.27 grams per minute). Blood pressure 90. Reflexes now slightly increased.

9.50 a.m.   Blood pressure 90. Reflexes distinctly increased. Pupils maximally dilated. Gave a few drops of ether, and the increased reflex excitability disappeared for about 10 minutes.

10.21½ a.m.   Seventh specimen. 0.5 gram in 30 seconds (1.0 gram per minute).

10.22 a.m.   Eighth specimen, 2.8 grams in 4 minutes (0.7 gram per minute).   Blood pressure 60.   Reflex excitability considerably increased.   Obtained indifferent blood from abdominal aorta.   Weight of the two adrenals 0.410 gram.

The second adrenal blood specimen, collected before administration of morphine, was assayed at 1:5,000,000, corresponding to an output of 0.00052 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute.

The fourth specimen, obtained 1 minute after intravenous injection of 10.2 mgm. morphine sulphate per kilogram was much stronger than 1:2,500,000, stronger than 1:1,250,000, decidedly weaker than 1:375,000, weaker than 1:500,000, not much different from 1:625,000 (confirmed by two sets of observations). (fig. 3, observations *26, 28, 30, 48,* and *50*).   Taking the fourth specimen at 1:600,000, we get 0.0027 mgm. per minute for the cat, or 0.0013 mgm. per kilogram per minute (5 times the initial output).

The sixth adrenal specimen, procured 24 minutes after injection of morphine, was not as strong as the fourth, the flow during its collection being somewhat greater (fig. 3, observations *22,* and *28*).   It was assayed at 1:800,000, giving 0.0028 mgm. per minute for the cat, or 0.0013 mgm. per kilogram per minute, the same as for the fourth specimen.

The eighth specimen, collected 66 minutes after morphine was given, was much the strongest.   Even when diluted with its own volume of indifferent blood, it was quite as strong as the fourth specimen, i.e., the concentration in the eighth was not less than 1:300,000.   It was decidedly stronger than 1:375,000. Diluted with one volume of indifferent blood, it was weaker than 1:500,000, i.e., the eighth was weaker than 1:250,000 (fig. 3, observations *30, 32, 34, 50* and *52*).   Taking the eighth specimen at 1:300,000, we get 0.0023 mgm. per minute as the epinephrin output for the cat, or 0.0011 mgm. per kilogram per minute (from 4 to 5 times the initial value).   With the high concentration reached it is possible that the blood flow was too small to permit the calculated output to equal what would have been attained with a larger flow.
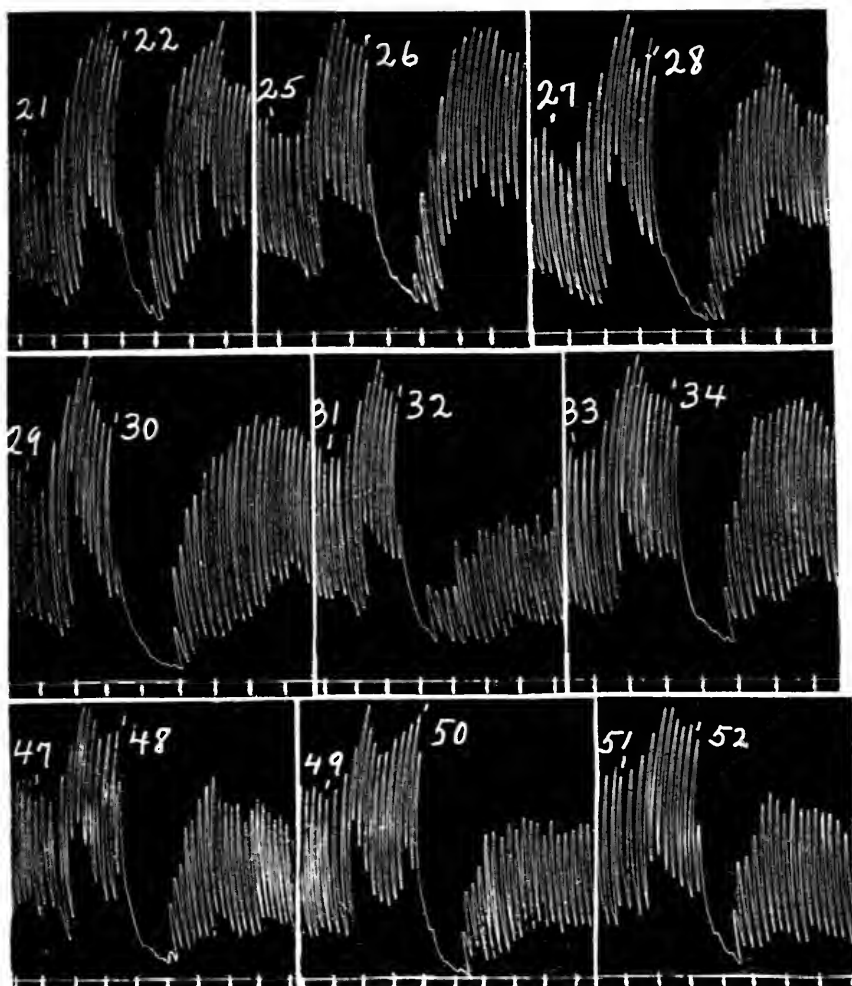
FIG. 3. INTESTINE TRACINGS: BLOODS FROM CAT 626

At *21, 25, 27, 29, 31, 33, 47, 49* and *51* Ringer was replaced by indifferent blood
(collected after administration of morphine) and this at *22* by the sixth adrenal
blood specimen (collected twenty-four minutes after administration of morphine),
at *26* by indifferent blood to which was added adrenalin to make a concentration
of 1:625,000, at *28* and *48* by the fourth adrenal blood specimen (collected one
minute after intravenous injection of morphine), at *30* by indifferent blood to
which was added adrenalin to make a concentration of 1:375,000, at *32* by the
eighth adrenal blood specimen (collected sixty-five minutes after administration
of morphine), at *34* and *52* by the eighth specimen diluted with one volume in-
different blood, and at *50* by indifferent blood to which was added adrenalin to
make a concentration of 1:500,000. All the bloods were diluted with three
volumes Ringer (the adrenalin bloods after adding the adrenalin, and the eighth
adrenal blood specimen after adding the indifferent blood). (Reduced to three-
fifths.)

67

The greatest increase observed in the series of cats was in cat 616, a male, weighing 2.71 kgm. The second specimen, taken before morphine (blood flow 4.4 grams per minute), had a concentration of 1:10,000,000 epinephrin, corresponding to 0.00016 mgm. per kilogram per minute. The fourth specimen, collected $2\frac{1}{2}$ minutes after intravenous injection of 9.2 mgm. morphine sulphate (with a blood flow of 1.5 gram per minute) had a concentration of 1:450,000, corresponding to an output of 0.0012 mgm. per kilogram per minute (about 7 times the initial value). The sixth specimen, procured 25 minutes after morphine administration (blood flow 2.07 grams per minute) had the same concentration as the fourth, namely, 1:450,000, corresponding to an output of 0.0017 mgm. per kilogram per minute (10 times the initial output). The eighth specimen, collected 71 minutes after injection of morphine (with a blood flow of 0.82 gram per minute), was decidedly weaker than the fourth and sixth, indicating that at this stage the increase in the rate of output was disappearing. It was assayed at 1:850,000, corresponding to an output of 0.00037 mgm. per kilogram per minute (about twice the initial value). Injection of morphine caused a marked fall in blood pressure (from 140 to 70 mm. of mercury), with inhibition of the heart and temporary cessation of respiratory movements. Artificial respiration was started, but soon discontinued. The reflexes were only slightly increased. There was salivation at first: the pads of the feet remained dry throughout. The dilation of the pupils under ether was somewhat increased by the morphine. The eye reflexes were well obtained. Only a few whiffs of ether were given after the morphine, as the animal was perfectly quiet. It should be noted that the increased output of epinephrin was obtained in this cat and the others, although from the conditions of the experiments (ether anesthesia, tying of the animal, etc.) the familiar manifestations of general excitement were absent. What effect would be produced on the output in a non-anesthetised cat is unknown.

To test the question whether deep anesthesia continued throughout the experiment would permit an increase to be manifested, observations were made on a cat under urethane.

Condensed protocol.   Cat 624; pregnant female: weight 2.395 kgm. (without
embryos 2.09 kgm.)

Anesthetised with urethane (4 grams by stomach tube). Indif-
ferent blood obtained. The embryos were removed before the pocket
was formed. The vagi were divided in the neck. Pocket completed
and cannula inserted at 9.45 a.m.

9.46½ a.m.   First specimen, 1.0 gram in 30 seconds (2.0 grams per
minute).

9.47 a.m.   Second specimen, 3.4 grams in 2 minutes (1.7 grams per
minute).

9.59 a.m.   Injected 10. mgm. morphine sulphate into jugular. Blood
pressure 81 mm. of mercury at beginning of injection.
It rose slightly and then fell to 68 mm.

10.00 a.m.   Injected 10 mgm. morphine into jugular. The pressure rose
slightly, and then fell to 68 mm., at which it remained.

10.01 a.m.   Third specimen (not weighed).

10.01½ a.m.   Fourth specimen, 3.1 grams in 4 minutes (0.8 gram per
minute). During collection of this specimen the animal
began to gasp and the blood pressure rose suddenly to 84
mm. Started artificial respiration at once. Blood pres-
sure fell to 65 mm., at which it remained steady.

10.15 a.m.   Blood pressure 54 mm. No eye reflexes were present
throughout the experiment.

10.26 a.m.   Fifth specimen, 0.4 gram in 60 seconds (0.4 gram per
minute).

10.27 a.m.   Sixth specimen, 1.85 gram in 6 minutes (0.31 gram per
minute). Blood pressure 56 mm. Obtained indif-
ferent blood from abdominal aorta. Weight of the two
adrenals 0.422 gram.

The second adrenal specimen, taken before administration of
morphine, was found to be stronger than 1:4,000,000 adrenalin,
stronger than 1:3,300,000; decidedly weaker than 1:2,000,000,
weaker than 1:2,700,000. It was assayed at 1:3,000,000, corre-
sponding to an output of 0.00023 mgm. per kilogram per minute.
The outputs are all calculated on the total bodyweight, including
the embryos.

The fourth specimen, collected 2 minutes after intravenous
injection of 8.3 mgm. morphine sulphate per kilogram, was much

stronger than the second, even when it was diluted with 1 and with 3 volumes of indifferent blood (fig. 4, observations *20* to *24*). Diluted with 3 volumes of indifferent blood, the fourth specimen was not weaker than 1 : 1,330,000 adrenalin, decidedly weaker than 1 : 1,000,000, and decidedly stronger than 1 : 2,000,000 (fig. 4, observations *24, 26, 28,* and *30,* confirmed by other obser- vations not reproduced). That is, the fourth specimen was not weaker than 1 : 330,000, decidedly weaker than 1 : 250,000, and decidedly stronger than 1 : 500,000.

Later on, the segment was atropinised, which much increased its sensitiveness. The fourth specimen, diluted with 9 volumes of indifferent blood, gave a very strong reaction, too strong for an exact assay. Diluted with 19 volumes of indifferent blood, it was stronger than 1 : 13,330,000, and not far from 1 : 5,400,000, i.e., the specimen was stronger than 1 : 660,000 and not far from 1 : 270,000. It was finally taken at 1 : 300,000, corresponding to an output of 0.0011 mgm. per kilogram per minute (about 5 times the initial rate.)

The sixth specimen (obtained 27 minutes after injection of morphine) was diluted with 5 volumes of indifferent blood for the assay. This dilution was weaker than 1 : 2,000,000 adrenalin, not quite as strong as 1 : 2,700,000, stronger than 1 : 4,000,000, i.e., the sixth specimen was weaker than 1 : 330,000, not quite as strong as 1 : 450,000, stronger than 1 : 660,000. Later, on the atropinised segment confirmatory results were obtained. The concentration of the specimen was taken at 1 : 500,000, corre- sponding to an output of 0.00025 mgm. per kilogram per minute, practically the same as the initial output. It must be noted that with the very small adrenal blood flow at the time the sixth specimen was collected, and the high concentration already reached, it could not be expected that the calculated output should show a great increase, even if with a larger flow an in- crease would have been shown at this time.

In the last cat (625) to be referred to, a very large dose of morphine (87.0 mgm. per kilogram) was administered subcuta- neously, and the increase in the epinephrin output was much the smallest in the series. This was associated with a narcotic effect
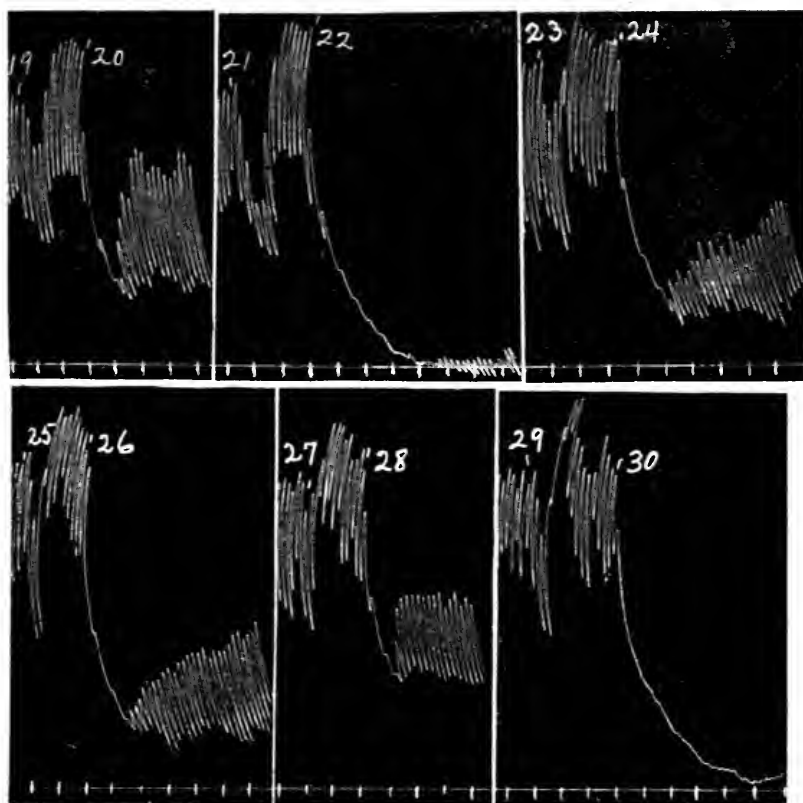
FIG. 4. INTESTINE TRACINGS; BLOODS FROM CAT 64

At *19* Ringer was replaced by indifferent (jugular) blood (collected before administration of morphine) and this at *20* by the second adrenal blood specimen (collected before administration of morphine). At *21, 23, 25, 27* and *29* Ringer was replaced by indifferent (arterial) blood (collected after administration of morphine) and this at *22* by the fourth adrenal blood specimen (collected one and one-half minutes after intravenous injection of morphine) diluted with one volume indifferent blood, at *24* by the fourth adrenal blood specimen diluted with three volumes indifferent blood, at *26* by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000, at *28* by indifferent blood to which was added adrenalin to make a concentration of 1:2,000,000, and at *30* by indifferent blood to which was added adrenalin to make a concentration of 1:1,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin, and the fourth specimen after adding the indifferent blood). (Reduced to one-half.)

of the drug more pronounced than with the smaller doses. In
this respect the animal approached more nearly to the dogs, which
showed either no increase of epinephrin output, or a very small
one.

Condensed protocol.   Cat 625; female; weight 2.295 kgm.

Under ether anesthesia inserted cannulae. Obtained indifferent
blood from the jugular.   Pocket completed at 9.53 a.m.

9.54½ a.m.   First specimen, 1.1 gram in 30 seconds (2.2 grams per
minute).

9.55 a.m.   Second specimen, 3.0 grams in 2 minutes (1.5 gram per
minute).

10.04 to 10.06 a.m.   Injected 200 mgm. morphine sulphate hypodermi-
cally (in 3 doses, 100, 50, and 50 mgm.).   The blood
pressure was 145 mm. of mercury before the injection,
rose slightly (to 153 mm.), and at the end of injection
had fallen to 128 mm.

10.10 a.m.   Blood pressure 114; cat quiet; respiration regular but
shallow; pads of feet dry; no salivation; no increased
reflexes; pupils nearly at maximum dilatation; nicti-
tating membranes forward.

10.15 a.m.   Blood pressure 104.   Condition of animal unchanged.
No ether needed.

10.19½ a.m.   Third specimen, 0.7 gram in 30 seconds (1.4 gram
per minute).

10.20 a.m.   Fourth specimen, 2.7 grams in 2½ minutes (1.1 gram per
minute).   Blood pressure 80 mm.

10.23 a.m.   Pupils maximal; eye reflexes now present; animal quiet;
a little ether given a few minutes later.

10.40½ a.m.   Fifth specimen (not weighed).

10.41 a.m.   Sixth specimen, 2.5 grams in 4 minutes (0.6 gram per
minute).   Blood pressure 66.   Obtained indifferent
blood from abdominal aorta.   Weight of the two ad-
renals 0.305 gram.

The second adrenal blood specimen, collected before the ad-
ministration of morphine, was stronger than 1:4,000,000, weaker
than 1:2,700,000 adrenalin (fig. 5, observations *4, 6* and *8*).
At *3, 5* and *7* Ringer's solution was replaced by indifferent blood

(obtained before injection of morphine), and this at *4* and *6* by indifferent blood to which had been added adrenalin to make up
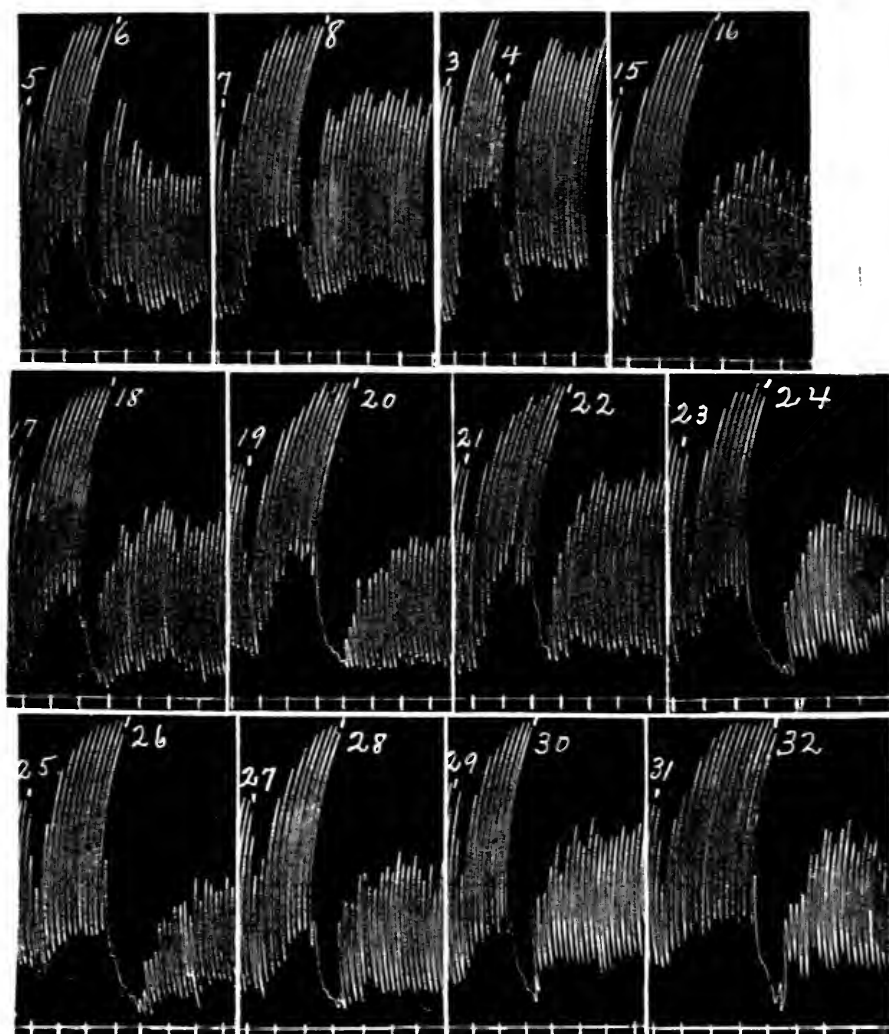

FIG. 5. INTESTINE TRACINGS; BLOODS FROM CAT 625

The description of these tracings is given in the text. (Reduced to one-half)

concentrations of 1:4,000,000 and 1:2,700,000 respectively. At *8* the indifferent blood was replaced by the second specimen.

As was the case throughout the experiment, all the bloods were diluted with three volumes of Ringer's solution before application to the segment, the adrenalin bloods after adding the adrenalin. The second specimen was assayed at 1:3,100,000, corresponding to an output of 0.00021 mgm. per kilogram per minute. The fourth specimen, obtained 15 minutes after hypodermic administration of 87 mgm. morphine sulphate per kilogram, was decidedly stronger than 1:2,700,000 adrenalin (tracing not reproduced), stronger than 1:2,000,000, weaker than 1:1,000,000, not much different from 1:1,330,000 (fig. 5, observations *16, 18, 20* and *22*). At *15, 17, 19*, and *21* Ringer's solution was replaced by indifferent blood (obtained after injection of morphine), and this at *18* by the fourth specimen, and at *16, 20* and *22* by the indifferent blood to which had been added adrenalin to make up a concentrations of 1:1,330,000, 1:1,000,000 and 1:2,000,000 respectively. The fourth specimen was taken at 1:1,400,000, corresponding to an output of 0.00035 mgm. per kilogram per minute (about one and a half times the initial output).

The sixth specimen, collected 35 minutes after the morphine injection, was much stronger than 1:2,000,000, not much weaker than 1:1,000,000 (fig. 5, observations *20* to *32*. At *23, 25, 27, 29* and *31* in figure 5, Ringer's solution was replaced by indifferent blood (collected after administration of morphine), and this at *24* by the sixth adrenal specimen, and at *26, 28*, and *30* by the indifferent blood to which adrenalin had been added to make up concentrations of 1:1,000,000, 1:1,330,000 and 1:2,000,000 respectively. At *32* the indifferent blood was replaced by the sixth specimen diluted with one volume of indifferent blood, The sixth specimen was stronger than 1:1,330,000 (observations not reproduced). Diluted with one volume of indifferent blood, it was weaker than the fourth specimen, i.e., the sixth was weaker than 1:700,000. This dilution of the sixth specimen was weaker than 1:1,330,000 (fig. 5, observations *28* and *32*) i.e., the sixth was weaker than 1:665,000. The dilution was stronger than 1:2,700,000 (tracings not reproduced), i.e., the sixth was stronger than 1:1,350,000. The dilution was not much different from 1:2,000,000, probably fully as strong (fig. 5, observations

30 and *32*), i.e., the sixth was not far different from 1:1,000,000. Taking it at 1:1,000,000, we get 0.00026 mgm. of epinephrin per kilogram per minute, about the same as the initial value.

Two typical experiments on dogs will now be given, one with subcutaneous, the other with intravenous injection of the drug.

Condensed protocol. Dog 622; male; weight 4.8 kgm.

Under ether anesthesia obtained indifferent blood (from jugular). Pocket completed at 10.05 a.m.

10.10½ a.m.   First adrenal specimen, 2.25 grams in 30 seconds (4.5 grams per minute).

10.11 a.m.   Second specimen, 4.58 grams in one minute.

10.13 a.m.   Finished hypodermic injection of 100 mgm. morphine sulphate. Blood pressure 138 mm. of mercury; no immediate change after injection.

10.20 a.m.   Blood pressure 92 mm. A little ether had been given from time to time, but after this no more was required.

10.25 a.m.   Blood pressure 98 mm. Traube-Hering waves, but not large.

10.28½ a.m.   Third specimen. 1.9 gram in 30 seconds (3.8 grams per minute).

10.29 a.m.   Fourth specimen, 4.1 grams in one minute Blood pressure 110 mm.   Eye reflexes present, but no other reflexes were elicited.

10.40 a.m.   Blood pressure 110 mm. Traube-Hering waves again present. Condition of animal unchanged.

10.58½ a.m.   Fifth specimen, 1.9 gram in 30 seconds (3.8 grams per minute).

10.59 a.m.   Sixth specimen. 5.175 grams in 90 seconds (3.45 grams per minute). Blood pressure 94 mm.

11.15 a.m.   Blood pressure 86 mm. The animal is quiet. The Traube waves have disappeared.

11.44½ a.m.   Seventh specimen. 1.5 gram in 30 seconds (3 grams per minute).

11.45 a.m.   Eighth specimen. 5.1 grams in 2 minutes (2.55 grams per minute). Blood pressure 73 mm. Obtained indifferent blood from abdominal aorta. Weight of the two adrenals 0.87 gram.

The second adrenal blood specimen, collected before morphine, was decidedly weaker than 1:2,500,000, weaker than 1:3,750,000, somewhat stronger than 1:5,000,000 (fig. 6, observations *20* to *24*). This was confirmed by a similar set of observations taken before the segment was atropinised and another set towards the end of the assay, in which it was more distinctly shown that the second specimen was somewhat stronger than 1:5,000,000, and markedly stronger than 1:6,600,000. It was taken at 1:4,700,000, corresponding to an output of 0.0002 mgm. per kilogram per minute.

The fourth specimen, procured 16 minutes after subcutaneous injection of 20.9 mgm. of morphine sulphate per kilogram, had a concentration of epinephrin much the same as that of the sixth and eighth specimens, collected respectively 46 minutes and 92 minutes after administration of the drug (tracings not reproduced). All three were much stronger than 1:5,000,000 adrenalin. The sixth and eighth specimens were proved by separate observations to be not much different from 1:2,500,000. All were much weaker than 1:1,250,000. The sixth specimen was then shown to be decidedly stronger than 1:3,750,000; in one set of observations it seemed to be slightly weaker than 1:2,500,000, and in the next set to be slightly stronger than 1:2,500,000. The tracings are all very similar to those in figure 6 from observation *52* on, at which point in the assay a few are reproduced as examples. The fourth specimen was then shown to be much stronger than 1:3,750,000, and at this point two sets of observations indicated that it might be somewhat stronger than the sixth. One of these sets is reproduced in figure 6 (observations *52* and *56*). The segment reacted to rather small differences in the neighborhood of these concentrations, and the difference between *52* and *56* does not in this case indicate a great difference in the concentration of the two specimens. The fourth specimen was found at this point to be much the same as 1:2,500,000, distinctly stronger than 1:3,100,000, decidedly weaker than 1:1,900,000 (fig. 6, observations *56*, *58* and *60*). The sixth specimen was distinctly stronger than 1:3,100,000 (observations *52* and *58*). The eighth specimen was decidedly weaker than
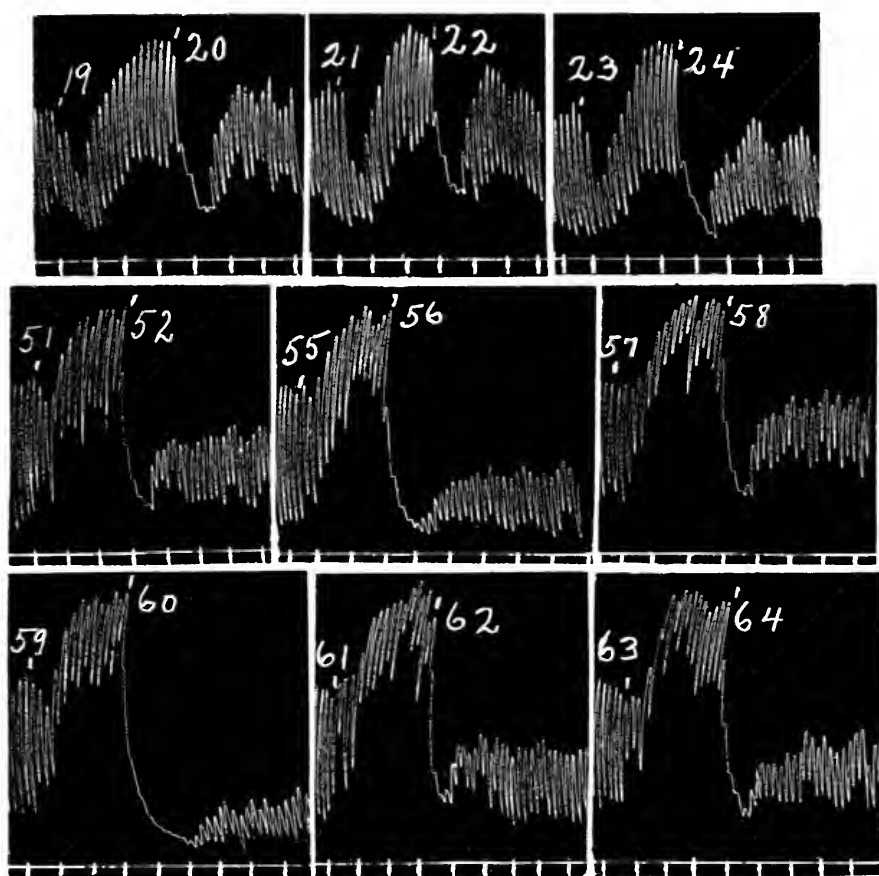
FIG. 6. INTESTINE TRACINGS; BLOODS FROM DOG 622

At *19, 21* and *23* Ringer was replaced by indifferent (jugular) blood (collected before administration of morphine) and this at *20* by the second adrenal blood specimen (collected before administration of morphine), at *22* by indifferent blood to which was added adrenalin to make a concentration of 1:5,000,000 and at *24* by indifferent blood to which was added adrenalin to make a concentration of 1:3,750,000. At *51, 55, 57, 59, 61* and *63* Ringer was replaced by indifferent (arterial) blood (collected after administration of morphine) and this at *52* by the sixth adrenal blood specimen (collected forty-six minutes after administration of morphine), at *56* by the fourth adrenal blood specimen (collected sixteen minutes after administration of morphine), at *58* by indifferent blood to which was added adrenalin to make a concentration of 1:3,100,000, at *60* by indifferent blood to which was added adrenalin to make a concentration of 1:1,900,000, at *62* by the eighth adrenal blood specimen (collected one and one-half hours after administration of morphine) and at *64* by indifferent blood to which was added adrenalin to make a concentration of 1:2,500,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to two-thirds.)

77

1:1,900,000, and not far different from 1:2,500,000 (fig. 6, observations *60, 62* and *64*).

Taking the fourth specimen at 1:2,500,000, we get 0.00034 mgm. per kilogram per minute as the output of epinephrin (somewhat more than $1\frac{1}{2}$ times the initial output). Taking the concentration of the sixth specimen at 1:2,700,000, slightly less than that of the fourth, we get 0.00026 mgm. per kilogram per minute, practically the same as the initial output. Taking the eighth specimen at 1:2,500,000 we get 0.0002 mgm. per kilogram, the same as before morphine administration. It is evident that in this dog practically no effect was caused by the drug upon the epinephrin output. The result was much the same in dog 623 with intravenous injection of morphine.

Condensed protocol. Dog 623; male; weight 6.6 kgm.

Under ether anesthesia obtained indifferent blood (from jugular). Completed pocket at 10.19 a.m.

$10.19\frac{1}{2}$ a.m.   First adrenal specimen, 3.9 grams in 30 seconds (7.8 grams per minute).

10.20 a.m.   Second specimen. 9.15 grams in one minute.

$10.23\frac{1}{2}$ a.m. Finished injection of 25 mgm. morphine sulphate into jugular. The injection occupied about 40 seconds. Blood pressure before injection, 122 mm. of mercury. It rose a little and then fell rapidly to 96 mm. a few seconds after the end of injection. There was marked slowing of the pulse.

10.24 a.m.   Third specimen. 2.1 grams in 30 seconds (4.2 grams per minute).

$10.24\frac{1}{2}$ a.m.   Fourth specimen. 5.0 grams in one minute. Blood pressure during collection of third specimen 100 mm., at end of collection of fourth, 110 mm.

10.30 a.m.   Heart much slowed, blood pressure 110 mm. Cut both vagi.

10.45 a.m.   Injected into jugular 30 mgm. morphine sulphate. The blood pressure fell from 110 to 66 mm. of mercury. Ether was given in small amount from time to time as required.

$11.06\frac{1}{2}$ a.m.   Fifth specimen. 3.4 grams in 30 seconds (6.8 grams per minute).

11.07 a.m.   Sixth specimen, 6.0 grams in one minute.   Blood pressure
             during collection of fifth specimen 120 mm., during
             collection of sixth 104 mm.
11.45½ a.m. Seventh specimen, 0.75 gram in 30 seconds (1.5 gram
             per minute).
11.46 a.m.   Eighth specimen, 3.9 grams in 3 minutes (1.3 gram per
             minute). Blood pressure 57 mm. Obtained indiff-
             erent blood from abdominal aorta. Weight of the
             two adrenals 0.82 gram.

The second adrenal blood specimen, collected before mor-
phine, was much stronger than 1:9,300,000 adrenalin, much
stronger than 1:6,660,000, stronger than 1:5,300,000, slightly
weaker than 1:4,000,000, and decidedly weaker than 1:2,700,000.
These results are illustrated in figure 7, in which Ringer's solution
was replaced at *17*, *19*, *21*, and *23* by indifferent blood, and this at
18, *20*, and *24* by indifferent blood to which had been added
adrenalin to make a concentration of 1:5,300,000, 1:6,660,000
and 1:4,000,000 respectively.   At *22* the indifferent blood was
replaced by the second specimen.   To save repetition, it may be
stated that in the whole assay the bloods were always diluted
with 3 volumes of Ringer's solution before application to the
segment, the adrenalin bloods after adding the adrenalin.   These
results were confirmed by several additional sets of observations,
some of which showed that the concentration of the specimen
differed little from 1:4,000,000. The concentration of epinephrin
in the second specimen was taken at 1:4,200,000, corresponding
to an output of 0.00033 mgm. per kilogram per minute.

The fourth specimen, taken one minute after intravenous in-
jection of 7.5 mgm. of morphine sulphate per kilogram, appeared
to be somewhat stronger than the sixth specimen, collected 44
minutes after administration of the drug, but both were too strong
for assay without diluting with indifferent blood.   In figure 7,
Ringer's solution was replaced at *27* and *29* by indifferent blood,
and this at *28* and *30* by the fourth and the sixth specimens
respectively.   The fourth specimen was now diluted with one
volume of indifferent blood.   This dilution was shown to be
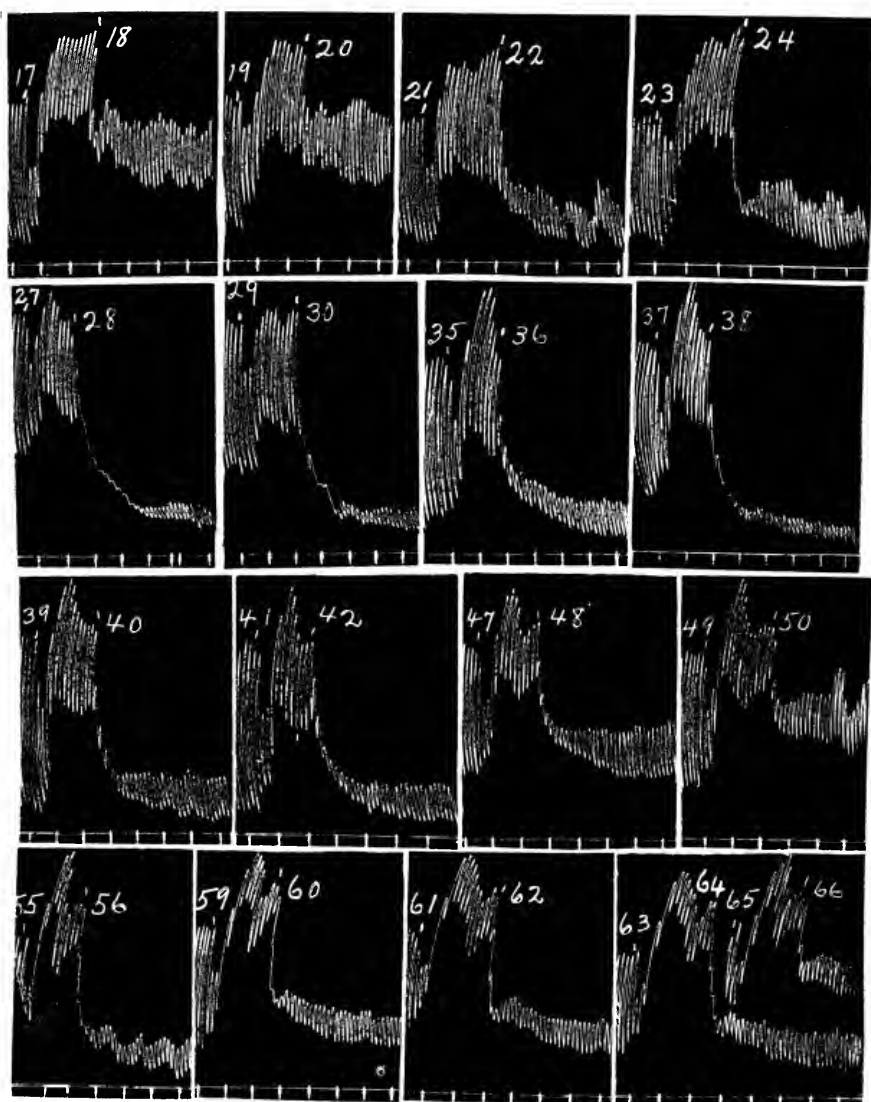weaker than 1:2,660,000, but stronger than 1:4,000,000, i.e.,

Fig. 7. Intestine Tracings; Bloods from Dog 623

The description of these tracings is given in the text. (Reduced to one-half)

the fourth specimen was weaker than 1:1,330,000 and stronger than 1:2,000,000 adrenalin. In figure 7, Ringer's solution was replaced at *35*, *37* and *39* by indifferent blood, and this at *36* by the fourth specimen (diluted with one volume of indifferent blood), and at *38* and *40* by indifferent blood to which had been added adrenalin to make up a concentration of 1:2,660,000 and 1:4,000,000 respectively. From these observations it follows that the fourth specimen was weaker than 1:1,330,000 and stronger than 1:2,000,000. The sixth specimen diluted with one volume of indifferent blood, did not differ greatly from the fourth specimen similarly diluted, as shown by comparing observations *42* and *36* (fig. 7). At *41* Ringer's solution was replaced by indifferent blood, and this at *42* by the sixth specimen diluted with one volume of indifferent blood. Other observations, not reproduced, confirmed these results. Some observations were now made in which the fourth and sixth specimens were diluted with 2 volumes of indifferent blood, and it was shown that each in this dilution was stronger than 1:6,660,000, and weaker than 1:4,000,000, i.e., the fourth and sixth specimens were both stronger than 1:2,200,000, and weaker than 1:1,330,000. For example, in figure 7, Ringer's solution was replaced at *47*, *49* and *55* by indifferent blood, and this at *48* by the sixth specimen (diluted with 2 volumes of indifferent blood), at *50* by indifferent blood to which had been added adrenalin to make up a concentration of 1:6,660,000, and at *56* by indifferent blood to which had been added adrenalin to make up a concentration of 1:4,000, 000. In other observations (not reproduced) it was shown that the sixth specimen, diluted with 2 volumes of indifferent blood was about as strong as 1:5,000,000, i.e. the sixth specimen was about as strong as 1:1,660,000. A series of observations was also made in which the fourth and sixth specimens were diluted with 3 volumes of indifferent blood. The fourth specimen thus diluted was distinctly weaker than 1:4,000,000, but not much weaker than 1:5,300,000, i.e., the fourth adrenal blood was not much weaker than 1:1,330,000, although distinctly weaker than 1:1,000,000. Similar results were obtained for the sixth specimen

diluted with 3 volumes of Ringer's solution, but it was again shown that it was somewhat weaker than the fourth specimen.

The fourth specimen was taken at 1:1,500,000, corresponding to an output of 0.0005 mgm. per kilogram per minute ($1\frac{1}{2}$ times as great as the output before morphine). The sixth specimen, assayed at 1:1,700,000, represented the same output as the fourth, namely 0.0005 mgm. per kilogram per minute. At this stage there might accordingly have been a small increase in the rate of output, but of a totally different order of magnitude from that caused by morphine in cats, or by strychnine in dogs and cats.

The eighth adrenal specimen, obtained 83 minutes after injection of morphine, gave so great a reaction that it was necessary to dilute it with 5 volumes of indifferent blood. This dilution was weaker than 1:4,000,000 adrenalin, much stronger than 1:6,600,000, and little different from 1:5,300,000, i.e., the eighth specimen was weaker than 1:660,000, much stronger than 1:1,100,000 and about the same as 1:870,000. This is illustrated in figure 7. At 55, 59, 61, 63 and 65, Ringer's solution was replaced by indifferent blood, and this at 60 and 64 by the eighth specimen, diluted with 5 volumes of indifferent blood; at 56, 62 and 66 by indifferent blood to which had been added adrenalin to make a concentration of 1:4,000,000, 1:5,300,000 and 1:6,600,000 respectively. Taking the eighth specimen at 1:875,000 we get for the rate of epinephrin output at this time 0.00025 mgm. per kilogram per minute, no more than the initial output.

In another dog (620), a male, weighing 6.4 kgm., the specimen taken before morphine (bloodflow 8.2 grams per minute) was assayed at 1:4,000,000, giving an output of epinephrin of 0.00032 mgm. per kilogram per minute.

A specimen of adrenal blood, collected 5 minutes after intravenous injection of 6.2 mgm. morphine sulphate per kilogram, had a high concentration of epinephrin, corresponding with the slow blood flow from the adrenals (1.5 gram per minute), due to the drop of blood pressure from 90 to 30 mm. of mercury caused by the morphine. The specimen was assayed at 1:500,000,

giving an output of 0.00047 mgm. per kilogram per minute (about $1\frac{1}{2}$ times the rate before administration of the drug).

An adrenal blood specimen obtained 43 minutes after injection of morphine (bloodflow 1.9 gram per minute, associated with a small recovery in the blood pressure) was assayed at 1:660,000, corresponding to an ouput of 0.00044 mgm. per kilogram per minute.   The small increase in the output following the injection of morphine therefore apparently continued at this time.

When the morphine was injected respiration ceased as the blood pressure reached its lowest point.   Artificial respiration was at once employed, and in about 2 minutes spontaneous respiratory movements began to return.   Salivation was the only symptom, except the apparently complete narcosis, which rendered it unnecessary to give ether after the injection.   The pupils were maximally dilated.   Reflex excitability was not increased.

In another dog (621), a female weighing 7.0 kgm., with a considerable amount of fat in the abdomen, a specimen of adrenal blood taken before morphine had a concentration of 1:5,000,000 (blood flow 8.25 grams per minute).   This gives an output of 0.00024 mgm. per kilogram per minute.   A specimen obtained 20 minutes after subcutaneous injection of 14.3 mgm. of morphine sulphate per kilogram, had a concentration of 1:3,500,000 (blood flow 6.7 grams per minute).   This gives an output of 0.0003 mgm. per kilogram per minute.   At this point no definite change had occured in the output.

A specimen collected 45 minutes after administration of the drug was taken at 1:1,300,000.   With the blood flow of 4.3 grams per minute, this corresponds to an output of 0.0004 mgm. per kilogram per minute.   A specimen procured 84 minutes after injection of morphine also gave an output of 0.0004 mgm. per kilogram per minute (with a blood flow of 3.5 grams per minute).   The response of the segment to successive applications of the same adrenal blood or of indifferent blood containing the same concentration of adrenalin was so inconstant, both before and after atropinisation, that the estimation of the concentrations was less satisfactory than usual.   No great stress

need therefore be laid on the apparent small increase in the output in the later specimens of adrenal blood. However, the increase is of the same order of magnitude as in the other dogs, and so nearly within the limits of error even with the best methods of bio-assay at present at our disposal, that it is impossible to attach any significance to it. In the cats it is quite different, and there can be no doubt about the increase.

It is common, or indeed the rule, to find that the response of a segment to a given concentration of epinephrin changes somewhat in the course of a long series of observations. Such changes are easily taken account of by repeating observations sufficiently, and only comparing observations which do not lie too far apart. But in our experience it is rare to find a segment which, so to say, contradicts itself from observation to observation.

The smallest dose of morphine used for the dogs was 2.6 mgm. per kilogram intravenously (in dog 680). No ether was required after the morphine. No detectable change was produced in the output of epinephrin.

As shown by Elliott (4), morphine causes a diminution in the store of epinephrine in the adrenal when its innervation has not been interfered with. It has often been assumed that such a depletion is a proof of increased output. This, however, is far from being the case. Strychnine, for instance, which causes a greater and possibly a more lasting increase in the output than morphine both in cats and dogs, does not produce any sensible depletion of the epinephrin store of the normally innervated adrenal of the cat as compared with its previously denervated fellow (5). The epinephrin store is simply a balance struck between production and liberation of epinephrin. It is not in itself a measure of the absolute magnitude of either. A diminution in the store could equally well be caused by a slackening in the upbuilding as by an increase in the output. Since, however, we have now demonstrated that in the cat morphine does for a time augment the output, this can obviously play a part in the depletion. In dogs, on the other hand, morphine also causes depletion of the store (2), and on the whole about as great a relative depletion as in cats. Since we have shown that the output in the dog is,

when increased at all, far less affected by the drug than in the cat, it seems logical to conclude that the depletion can be due also to diminution in the rate of upbuilding and storage.

SUMMARY

Morphine, administered subcutaneously or intravenously, causes in cats an increase in the rate of output of epinephrin from the adrenals. As much as 10 times the initial rate has been observed. The animals were anesthetised with ether (in one experiment with urethane) before the morphine was administered, and therefore it is not known what increase may be caused in the absence of these anesthetics, which do not themselves appear to increase the output. The symptoms produced by morphine in non-anesthetised cats cannot be due, in any important measure to an increased output of epinephrin, since they are all obtained, and apparently in undiminished intensity, in cats after removal of one adrenal and the chief part of the other, and denervation of the remaining fragment.

In dogs either no increase in the output of epinephrin or a very slight one was caused by morphine. This difference in the action of the drug in the two animals is as marked as the other pharmacological differences.

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THE INFLUENCE OF MUSCULAR EXERCISE ON NORMAL CATS COMPARED WITH CATS DEPRIVED OF THE GREATER PART OF THE ADRENALS, WITH SPECIAL REFERENCE TO BODY TEMPERATURE, PULSE AND RESPIRATORY FREQUENCY

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It is widely held that muscular weakness is a symptom of adrenal insufficiency, and often assumed that interference with the epinephrin output is the factor essentially responsible for the muscular weakness. If either or both of these assumptions are correct, it might be thought likely that repeated spells of severe muscular exertion in animals with only enough adrenal tissue left to maintain life, would suffice to develop a physiological insufficiency revealed by some difference in susceptibility to, or in the rapidity of recovery from fatigue. If the second assumption is correct, animals whose epinephrin output has been greatly diminished (or abolished) without reduction of the total adrenal tissue to the necessary minimum (as in cats after removal of one gland and section of the nerves of the other) ought to react differently from normal cats to strenuous muscular exercise.

It may be said at once that no difference was observed between the normal animals and those which had recovered after removal of a large proportion of the adrenal tissue, presumably involving a marked diminution in the epinephrin output, or those whose epinephrin output had been interfered with by section of the nerves. The results, as regards survival, of partial adrenalectomy on 18 cats have already been published (1). Five of the cats, which had completely recovered and were indistinguishable in their behavior from the normal animals, were used for the experi-

87

ments on muscular exercise. They were caused to run either on a form of treadmill or in a revolving cage. The rectal temperature, pulse rate and rate of respiration were taken before and after the spell of exercise. No attempt was made to cause the animals to perform an equal amount of work in the various experiments, but in all a considerable, in some an extreme, degree of fatigue was induced. It was not believed to be possible to demonstrate small differences, if such exist, in this way, nor was it supposed that small differences could have much significance for the question at issue. For this reason the idea of attempting a comparison of the metabolism in normal and operated animals was abandoned, as soon as it was seen that the simple tests instituted revealed no clear difference. A summary of the results of observations on 4 control cats (514 a male; 513, 515 and 516 females) and 6 operated cats are given in table 1. The room temperature did not vary more than about a degree during an experiment. The numbers in the last column of the table are the averages of the room temperature readings made throughout each experiment.

Cat 489, a female, had the left adrenal and one-third of the right excised, on December 21, 1920, fifty-six days before the experiments on exercise were begun. Its weight was then 2.4 kgm. On January 8, 1921, it is noted that the animal appeared well. Rectal temperature 36.5°C., pulse 188, respiration 36. At the time of the first experiment (February 15) it weighed 2.59 kgm., and was in good condition. Seventeen days after the second experiment (on March 8) it weighed 2.65 kgm., and was in excellent health. Its condition was not affected by the exercise experiments, and its weight on May 6 was 2.66 kgm.

Cat 498, a female, weighing 1.6 kgm., had three-fifths of the right adrenal removed on January 10, and the whole of the left adrenal on January 28. It had regained its weight and was indistinguishable in its behavior from a normal cat when the exercise experiments were begun on February 9. On February 10, it weighed 1.9 kgm. and on March 8, after completion of the exercise observations, 1.98 kgm. It remained in excellent condition and was afterwards employed for other experiments, which will be mentioned in another place. On April 19, it weighed 2.36 kgm., and on May 27, 2.22 kgm.

TABLE 1

CAT	DATE	EXERCISE	RECTAL TEMPERATURE	HEART BEATS PER MINUTE	RESPIRATIONS PER MINUTE	ROOM TEMPERATURE
		minutes	°C			°C.
513	February 10		38.4	208		26.5
		5	39.1	248	*	
514	February 10		39.3	242		25.5
		10	40.4	276	*	
	February 11		39.1	220	56	24.5
		3†	39.9	256	60	
515	February 11		38.9	240	48	25.0
		5	39.6	272	62	
516	March 8		38.8	184	24	25.5
		11	39.6	‡	246	
489	February 15		38.7	228	34	22.2
		8	39.3	‡	45	
	February 19		37.4	274	32	22.2
		15	38.3	340	45	
	March 25		38.2	198	28	25.0
		25**	39.9	296	320	
498	February 9		39.1	230	32	25.0
		36**	39.6	‡	36	
	February 14		38.7	186	34	21.1
		10**	38.5	270	62	
	February 16		37.8	262	34	27.5
	11 a.m.	9	38.6	296	98	
	3.30 p.m.		39.6	248	58	26.5
		9	40.2	316	92	
	February 24		38.1	164	42	20.0
		37	38.4	280	72	
499	February 14		36.9	196	40	21.1
		6	37.3	228	64	
	February 16		38.4	178	58	27.5
	11.15 a.m.	9	38.5	300	91	
	3.50 p.m.		38.9	176	50	
		8	39.6	320	78	
	February 23		38.0	132	50	23.3
		45	38.7	244	96	
501	February 15		38.2	208	24	22.2
		5	39.4	260	71	
	February 19		37.6	184	37	21.1
		25	38.4	‡	52	
	March 3		38.2	206	34	24.5
		12	39.1	284	92	

TABLE 1—*Continued*

CAT	DATE	EXERCISE	RECTAL TEMPERATURE	HEART BEATS PER MINUTE	RESPIRATIONS PER MINUTE	ROOM TEMPERATURE
		minutes	°C.			°C.
505	February 15		38.5	181	28	22.2
		7	39.0	280	32	
	February 16		40.0	128	37	24.5
		6	40.3	200	36¶	
	February 19		38.3	132	36	22.2
		18	39.4	184	37¶	
	March 1		37.0	156	34	21.1
		30	38.0	188	32¶	
	March 8		38.9	144	42	25.5
		6	39.7	224	56¶	
	April 6		38.6	136	32	24.5
		20	39.8	208	140	
		22	40.2	244	188	
517	March 25		39.0	146	26	23.3
		60**	39.1	244	160	
	April 5		39.1	148	48	24.9
		15	40.4	256	164	
		25	39.7	184	124	

* Respiration faster and deeper.
† Refused to run longer.
‡ Heart too fast to count.
¶ Respiration deeper.
** Much fatigued.

Cat 499, a male, weighing 2.25 kgm., had two-fifths of the right adrenal removed on January 10, and the whole of the left gland on January 28. On February 10, the weight was 2.73 kgm. and the animal was in excellent condition. No harmful effect whatsoever was produced by the exercise experiments, and on March 8 the weight was 2.99 kgm. On April 27, the remaining part of the right adrenal was denervated (the major and minor splanchnics being cut and most of the semilunar ganglion excised). The animal weighed 3.17 kgm., and nine days thereafter, 3.05 kgm. Later it was used for an experiment with morphine, which will be mentioned in the next paper.

In cat 501, a female, weighing 2.19 kgm., a third to two-fifths of the left adrenal was removed on January 10. and on February 1, the whole of the right gland. On February 10, the animal weighed 2.34 kgm. and on March 8, after the experiment on exercise, 2.33 kgm. It was in excellent health, and on April 25 the remnant of the left adrenal

was denervated. The weight of the animal at this time was 2.77 kgm. and on May 4, 2.51 kgm. It was afterwards used for an experiment with morphine, described in the next paper.

In cat 505, a female, weighing 2.33 kgm., more than nine-tenths of the right adrenal (as was judged) was excised on January 18, and the artery tied. On February 3, two-thirds to three-fourths of the left adrenal was removed. On February 10, the animal weighed 2.39 kgm. and was in good condition. On six occasions thereafter it was put in the treadmill or the revolving cage. On the last day (April 6), a period of twenty minutes was succeeded by a second period of twenty-two minutes till a clonic convulsion occurred, and complete fatigue was induced. There were no harmful effects. The animal remained in the same excellent health, and weighed 2.7 kgm. on April 19, when it was employed for an experiment with morphine (next paper). On May 6 the weight was 2.74 kgm. The remnant of the left adrenal was denervated by section of the major and minor splanchnics and removal of most of the semilunar ganglion. Like the other cats subjected to this operation, it recovered without symptoms.

In cat 517, a female, the right adrenal was excised and the left denervated on February 18. On March 8, the original weight (1.88 kgm.) had been regained. On March 25, it weighed 2.05 kgm. and was in excellent condition. In the last exercise experiment it ran steadily in the cage for the first fifteen minutes. But during the second 25 minutes turn it ran no more than five minutes, sliding most of the time. It showed no harmful effects at all from the exercise. On April 11, it weighed 1.82 kgm., and on May 6, 2.02 kgm. On May 27, more than three-fourths of the left adrenal was excised. The animal recovered perfectly, without symptoms.

It would be impossible, we believe, to find in these experiments any support for the idea, that cats with a great anatomical deficiency of adrenal tissue are in any way handicapped as regards their ability to support and to recover from the effects of severe prolonged and repeated spells of muscular exertion. No observer, we think, could in this respect have distinguished the operated from the control cats. Considerable, sometimes extreme excitement accompanied the exercise, but neither in the degree and nature of the excitement nor in the associated reactions did any difference appear between the two groups of animals.

Boinet (2) also found that rats which had survived double adrenalectomy for five to seven months withstood extreme muscular exertion quite well. Practically there was no difference between them and normal rats.

We performed five experiments in cats, to determine whether in prolonged muscular exertion there is any definite depletion of the epinephrin store of an adrenal normally innervated, as compared with its previously denervated fellow. It was found that only when muscular exertion was carried to the possible limit was a definite depletion produced. Even very considerable fatigue, stopping short of this, may be associated with complete equality in the stores of the two glands. The experiment is a complex one, for great excitement accompanies the muscular exercise. We have shown, however, that excitement as such, at least such as is caused when a dog is allowed to bark at a cat for several hours without being able actually to touch it, does not cause a definite depletion (3). When convulsions occurred a possible influence of asphyxia is not excluded. The results of the five experiments follow.

Cat 519; female; weight 1.86 kgm. The left adrenal was denervated nineteen days before the experiment. The semilunar ganglion was excised, also one ganglion of the sympathetic chain just below the diaphragm and a portion of the chain. Recovery from the operation was satisfactory, but mange developed on the ears and head.

Rectal temperature 39.2°C., pulse 228, respiration 47 a minute. Room temperature varied from 23.3° to 24.4°C. The cat was placed in the revolving cage and ran well for seven minutes, when it began to show fatigue. Pupils widely dilated; erection of hairs of tail. Periods of rest were now alternated with periods of running for one hour. The animal seemed pretty thoroughly fatigued. Rectal temperature 37.7°C., pulse 284, respiration 82.

Left adrenal weighed 0.134 gram and contained 0.16 mgm. epinephrin.

Right adrenal weighed 0.130 gram and contained 0.16 mgm. epinephrin.

Cat 520; male; weight 3.58 kgm. Left adrenal denervated twenty-four days before the experiment, the semilunar ganglion being excised,

and one of the ganglia of the sympathetic chain below the diaphragm. The condition of the animal was excellent.

March 14, 11.15 a.m. Rectal temperature 38.7°C., pulse 166, respiration 34 to 36. Room temperature 23.3° to 24.4°C. Put into revolving cage for twenty-one minutes. Pupils wide. Eyes staring, tail hairs erect. Fatigued. Pads of feet wet. Rectal temperature now 40.1°C., pulse (bounding) 176, respiration 290. A few minutes later, pulse 184.

March 15, 4.00 p.m. Temperature 38.4°C., pulse 160, respiration 32. Room 27.8°C. Worked the animal in the cage off and on for one hour. Temperature now 40.6°C., pulse 160, respiration over 300 a minute.

March 16, 2.00 p.m. Temperature 38.3°C., pulse 144, respiration 24. Room 24.4°C. Two hours in cage. Quite fatigued. Temperature, 39.8°C; pulse 276, respiration 122.

March 17, 9.15 a.m. Temperature 38.3°C., pulse 200, respiration 24. Room 24.4°C. Constant work for 1 hour and 50 minutes in the cage. Temperature now 40.3°C., pulse between 300 and 350, respiration about 340. Weight 3.36 kgm.

Left adrenal weighed 0.140 gram and contained 0.14 mgm. epinephrin.

Right adrenal weighed 0.178 gram and contained 0.11 mgm. epinephrin.

Cat 547; female; weight 2.81 kgm.

March 17. Denervated left adrenal.

April 6. Weight 2.85 kgm. Temperature 38.7°C., pulse 208, respiration 40. Room 24.3°C. Ran 15 minutes in cage; eyes wide, hair up. Temperature now 39.8°C., pulse too fast to count, respiration 246. Returned to cage for 25 minutes more (only 10 minutes steady running). Much fatigued. At the end of the period it had a convulsion of the same character as that described in cat 548. Temperature now 40.6°C., pulse apparently over 350 (too rapid to count), respiration 240 to 250 a minute.

April 7. Temperature 40.3°C., pulse 212, respiration 40. Room 26.7°C. Put into cage for 20 minutes. Pretty well fatigued after 8 minutes, and did not run steadily thereafter. Temperature now 41.6°C., pulse 278, respiration 216. Pads of feet wet; hair up.

April 8. Temperature 40.8°C., pulse 230 to 240, respiration 28. Room 24.4°C. Put into cage for 1 hour, but was soon fatigued and did not run steadily for more than a short part of the time. Temperature

now 40.2°C., pulse very rapid and could not be counted; respiration 214.

Left adrenal weighed 0.162 gm. and contained 0.21 mgm. epinephrin.

Right adrenal weighed 0.151 gm. and contained 0.11 mgm. epinephrin.

Cat 548; male; weight 2.32 kgm.

March 17. Left adrenal denervated.

April 6. Temperature 38.3°C., pulse 182, respiration 56. Room 23.3°C. Ran twenty minutes in cage. Temperature now 40.7°C., pulse 280 to 300, respiration 224. Returned to cage. Ran fairly well for fifteen minutes. Six minutes later, the animal suddenly had a tonic convulsion, quickly followed by clonic convulsions. It frothed at the mouth and urinated. The convulsions lasted altogether for half a minute to a minute. Eyes wide, hair on tail very erect. The temperature was now 40.3°C., pulse too fast to count, respiration 240.

April 7. Temperature 39.3°C., pulse 196, respiration 48. Room 25.5°C. Put into cage for 25 minutes, then began to tire. Temperature 40.7°C., pulse 280, respiration 260. Returned to cage and worked slowly but constantly for 45 minutes. Pads of feet very wet. Temperature 41.2°C., pulse about 360, respiration over 300.

April 9. Temperature 39.7°C., pulse 186, respiration 40. Worked for 45 minutes in the cage, then rested for 65 minutes, and worked again for 20 minutes.

Left adrenal weighed 0.342 gram and contained 0.28 mgm. epinephrin.

Right adrenal weighed 0.324 gram and contained 0.20 mgm. epinephrin.

Cat 549; female; weight 2.37 kgm.

March 17. Denervated left adrenal.

April 11. Weight 2.0 kgm. Temperature 37.6°C., pulse 128, respiration 56. Room 22°C. Ran in cage for 30 minutes. Temperature now 37.7°C., pulse 244, respiration 250 to 260. Returned to cage, where it ran ten to twelve minutes; a convulsion occurred lasting two to three minutes, first tonic then clonic; eyes maximally dilated, erection of hairs very marked; reflexes exaggerated. Temperature now 37.1°C., pulse 180, respiration over 300. An hour's rest was then given, and the animal took some milk. Temperature now 35.0°C., pulse 198, respiration 60. It was then run for thirty to forty minutes in the cage, with intervals of rest. The temperature was 32.5°C., pulse 162 (feeble), respiration 270 to 280. Killed by a sudden blow.

Left adrenal weighed 0.110 gram and contained 0.13 mgm. epinephrin.

Right adrenal weighed 0.114 gram and contained 0.08 mgm. epinephrin.

SUMMARY

Normal cats and cats after removal of the greater portion of the adrenal tissue, or removal of one adrenal and interference with the epinephrin output of the other by section of its nerves, were subjected to prolonged and repeated spells of muscular exercise. No difference which could be attributed to interference with the adrenals was made out in the behavior of the animals as regards resistance to, or recovery from fatigue, or as regards the changes in rectal temperature, pulse or respiratory rate.

After severe muscular exertion a definite, although not a very great depletion of the epinephrin store of an adrenal with its innervation intact, as compared with its previously denervated fellow, may be observed. But even after considerable exertion falling short of great fatigue, no depletion may be present.

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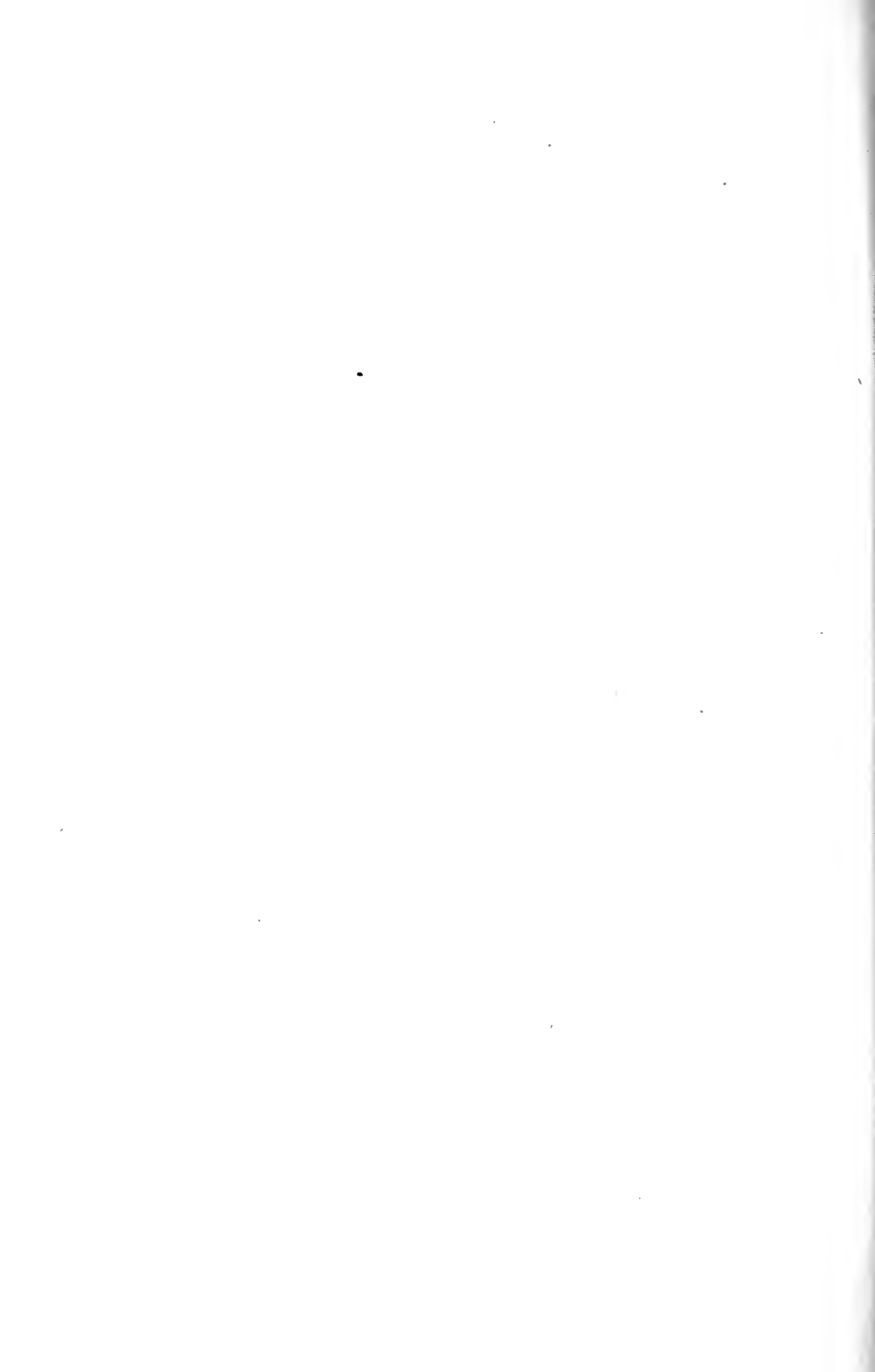
THE INFLUENCE OF MORPHINE ON NORMAL CATS AND ON CATS DEPRIVED OF THE GREATER PART OF THE ADRENALS, WITH SPECIAL REFERENCE TO BODY TEMPERATURE, PULSE AND RESPIRATORY FREQUENCY AND BLOOD SUGAR CONTENT

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Having failed to demonstrate any clear difference in the reactions to muscular exercise of normal cats and of cats deprived of the greater portion of the adrenal tissue (or of one adrenal with denervation of the remaining gland) (1), we tried whether the combination of morphine and enforced exercise would reveal any difference. It was supposed that since morphine causes excitation, accompanied by more or less continuous movements in cats, a further handicap might be placed upon animals deprived of the adrenals. No difference was found. But few experiments were made since it was at once seen that the marked hyperthermia, produced by morphine in the adrenalectomised cats, as in the normal animals, would preclude a further increase of temperature by the exercise. Unless otherwise stated, the morphine was always given hypodermically, and always the sulphate.

Thus, cat 498 (weighing 2.36 kgm.) from which $\frac{3}{5}$ of the right and the whole of the left adrenal had been removed about three months previously, and which had been used for exercise experiments described in the preceding paper (1), received 20 mgm. of morphine at 11.05 a.m. At 11.15 a.m. it began to exhibit symptoms of uneasiness (at 11.25 defecated), and these went on increasing till 12.25 p.m. The eyes were wide and the pupils dilated. At 2.00 p.m. the rectal temperature was taken before placing the cat in the revolving cage. It was 42.1°C. After seven to eight

97

minutes in the cage the temperature was 42.0°C. The cat was
not returned to the cage. At 3.00 p.m. it seemed still very rest-
less. At 5.00 p.m. it was quieter, and the temperature was 39.9°C.
The room temperature was 23.5° to 24.5°C. Next morning at
11.00 a.m. the temperature was 38.4°C.

In another cat (505), weighing 2.7 kgm., also employed in the
exercise experiments, $\frac{9}{10}$ of the right and $\frac{2}{3}$ to $\frac{3}{4}$ of the left adrenal
had been excised two and one-half to three months previously.
At 11.05 a.m. 20 mgm. of morphine was injected. The usual
symptoms developed, but the animal was quieter throughout than
cat 498. At 2.00 p.m. the rectal temperature was 41.7°C. After
six minutes exercise in the cage, the temperature was 42.3°C.
The animal was not placed in the cage again. At 5.00 p.m. the
temperature was 41.65°C. The cat was more restless than at
first. The room temperature was the same as in the last experi-
ment. Next morning at 11.00 a.m. the rectal temperature was
39.15°C.

It may be stated at once that no difference was made out be-
tween the adrenalectomised cats and normal animals as regards
their general behavior after morphine, or as regards the rise of
temperature, or changes in the pulse or respiratory frequency.
Considerable variations occurred in the different animals in the
intensity of the symptoms and also in the degree of hyperthermia.
But the characteristic symptoms and a rise of rectal temperature
were observed in every cat in the whole series of experiments, with
one exception, an animal which had been brought to the labora-
tory in a starving condition. Our experience is therefore some-
what different from that of Straub (2), who says that not all cats
exhibit excitement after morphine and that he has had several
which did not react at all, not showing even dilatation of the pupil.
He does not mention the extremely characteristic hyperthermia at
all. We agree with him that the symptoms suggest hallucination,
rather than "fright," still more than "rage." Not seldom the
animals seem willing to be even playful. Drunk rather than
frightened would be our interpretation. Straub's explanation
is that morphine is a very weak cerebral narcotic for these animals
producing only the first stage of narcosis, which with ether, etc., is
spoken of as the stage of excitation—a stage of cerebral disorder.

It did not appear in our observations that there was any very close relation between the degree of excitement (and the amount of movement) and the rise of rectal temperature.   A much greater amount of exercise in the revolving cage, without morphine, was generally associated with a smaller rise of temperature.

THE MORPHINE HYPERTHERMIA IN NORMAL CATS

The course, duration and magnitude of the hyperthermia, and its relation to the dose can be best described by giving in condensed form a few pertinent extracts from the protocols.

In cat 594, a normal female, weighing 2.7 kgm., 20 mgm. of morphine was injected at 11.05 a.m.   The animal vomited at 11.07, and at 11.20 symptoms of uneasiness were beginning and the pupils were wider.   At 11.50 it was very restless.   At 2.00 p.m. the rectal temperature was 40.5°C., and after ten minutes exercise in the cage 41.5°C. Room temperature 22°C.   At 3.00 p.m. the animal was still very restless.   At 5.00, it had quieted considerably and the rectal temperature was 39.65°C.   Next day at 11.00 a.m., 38.2°C.

In cat 595, a normal female, weighing 3.37 kgm., the rectal temperature at 10.50 a.m. was 38.3°C. (pulse 202, respiration 32).   It now received 40 mgm. morphine.   The symptoms were already evident at 11.33, a.m., when the temperature was 39.05°C.   Room temperature 25.5°C.   At 12.30 p.m. the animal was very active; rectal temperature 40.95°C.   At 1.35 p.m. it was very active, turning somersaults and panting; temperature 41.2°C.   At 3.00 p.m. it was quieter, and the temperature was 40.3°C.   At 4.00 p.m., still quieter; temperature 39.45°C. (pulse 220, respiration 36).   At 9.30 a.m. next day, it was apparently normal and took milk, but the temperature was still 39.45°C. (pulse 220 to 240, respiration 24).

In a few experiments the excitement due to the morphine culminated in a convulsion.   Thus in cat 597, a female, weighing 2.3 kgm., the rectal temperature at 11.00 a.m. was 37.8°C. (pulse 228, respiration 56).   Room temperature 25.5°C.   Morphine (30 mgm.) was now given. In twenty minutes restlessness and apparent apprehension began to be noticeable.   At 11.38 a.m. the animal was quite restless; temperature 39.7°C.   From 11.45 to 11.55 a.m. it jumped, rolled and salivated. At 12.15 p.m. it had a convulsion lasting one to two minutes, and then became more quiet.   At 12.34 p.m. the temperature was 41.4°C. and

the animal was quiet. At 1.45 p.m., temperature 40.8°C. (pulse 250, respiration 80 to 90); at 3.00 p.m. 40.3°C.; at 4.00 p.m. 39.15°C. (pulse 208, respiration 56); animal quiet. Next day at 9.30 a.m., temperature 38.9°C. (pulse 240, respiration 38).

The next experiment is an instance in which there was at no time even moderately increased muscular activity. Yet the rise of rectal temperature and the maximum reached were as great as in many animals showing marked activity. Such observations confirm the conclusion already drawn from a comparison of the morphine and exercise experiments, that the morphine hyperthermia is not primarily, or at least not solely due to increased muscular activity.

Cat 598, female; weight 2.275 kgm. At 11.05 a.m., the rectal temperature was 39.2°C.,room 25.5°C.; pulse 184; respiration 42. Injected 30 mgm. morphine. At 11.07 a.m. the cat vomited. At 11.30 a.m. it was quiet, the pupils dilated; temperature 39.6°C. At 12.20 p.m. quiet, but seems apprehensive. At 12.37 p.m., temperature 41.0°C.; the cat occasionally "paws" but sits all the time in a corner as if "drunk;" no change in behavior at 12.50 p.m. At 1.48 p.m., temperature 41.7°C.; cat still quiet. At 2.45 p.m., occasionally walks around in the cage. At 3.00 p.m. temperature 41.7°C.; seems a little more uneasy and apprehensive. At 4.00 p.m. temperature 41.55°C.; pulse 224, respiration 85; behavior the same as at 3.00 p.m. Next morning, temperature 38.4°C.; pulse 220, respiration 36; animal seemed normal and took milk.

In the next two experiments (cats 600 and 601) the effect of a much smaller dose (about 1.5 mgm. per kgm) was tried. The symptoms were more marked, especially in cat 600, than in cat 598, but the rise of temperature was much less.

Cat 600, female; weight 2.51 kgm. At 11.00 a.m. rectal temperature 38.0°C.; room varied from 21°C. to 21.5°C. At 11.03 a.m. morphine (4 mgm.). At 11.35 a.m. no symptoms; temperature 38.2°C. At 11.55 a.m. the cat defecated; seemed uneasy. At 12.10 p.m., temperature 38.9°C., a little more active. At 12.30 p.m. quite restless. At 12.47 p.m. very excitable, especially to noise; temperature 39.6°C. At 1.38 p.m. apprehensive and excitable to noise, but otherwise quiet;

temperature 39.9°C., the maximum observed. Thereafter, 39.8°C. (2.10 p.m.), 39.8°C. (3.03 p.m.), 39.7°C. (4.03 p.m.), 39.3°C. (5.30 p.m.). Now quiet. Next day at 9.00 a.m. 38.2°C.; animal normal and took milk.

In cat 601, a female, weighing 2.82 kgm., 4 mgm. of morphine was given at 11.06 a.m. Temperature just before, 38.45°C., and thereafter 38.55°C. (11.33 a.m.), 39.2°C. (12.13 p.m.), 39.45°C. (12.50 p.m.), 39.9°C. (1.40 p.m.), 39.9°C. (2.18 p.m.), 39.7°C. (3.05 p.m.), 39.1°C. (4.05 p.m.), 39.2°C. (5.30 p.m.). The animal began to appear uneasy and restless at 11.50 a.m. It did not move about much throughout the experiment although it seemed apprehensive and somehat restless even at the end. The room temperature varied from 21°C. to 21.5°C. Next day at 9.00 a.m. the animal was normal (temperature 37.95°C.), and took milk.

Only one animal in the series died, presumably from the morphine. This was cat 596, a female, weighing 1.795 kgm. At 10.55 a.m. its temperature was 38.3°C., pulse 188, respiration 52. Room 25.5°C. At this time it received 20 mgm. morphine. At 11.30 a.m. there was very little restlessness, and the temperature was 38.9°C. At 12.32 p.m. the temperature was 41.45°C., and the cat was moderately active and apprehensive. At 1.40 p.m. it was quiet, but moved about occasionally; temperature 41.7°C., pulse over 300, respiration about 300 a minute. At 3.05 p.m. not much change in its condition, temperature 41.7°C. At 4:00 p.m. temperature 40.15°C.; heart irregular, weak and very rapid, respiration 120. The animal was quiet, or "pawing" occasionally. It died during the night.

One experiment was made with intravenous injection of morphine. Except for the more rapid onset of the symptoms and the more prompt increase in temperature, the result was the same as with subcutaneous administration. It is noteworthy, that with a dose of less than 5 mgm. of morphine sulphate per kgm., the rectal temperature rose over 1°C. in 15 minutes, and 2.7°C. in 35 minutes.

Cat 617; female; weight 2.16 kgm. At 10.45 a.m. the temperature was 38.4°C., pulse 202, respiration 71. At 10.50 a.m. 10 mgm. of morphine was injected intravenously. In 20 to 30 seconds there was

profuse salivation, the heart beat became more forcible and slower, the pupils more dilated, the hair on the tail and back erect. When placed in a cage the cat at once began to run around restlessly, although she was quiet (or dull) when tied on the board. At 11.05 a.m. the temperature was 39.5°C., pulse 164, respiration 36 to 40; at 11.25 p.m. 41.1°C., 126 and 40 to 42. Salivation was not now marked, but the pilomotor effect was still obvious, and the cat was very restless. At 12.00 m. the temperature was 42.05°C. pulse 156, respiration 58; and at 1.00 p.m. temperature 42.2°C., pulse and respiration unchanged; general condition of the animal the same. At 3.00 p.m. the temperature was 41.3°C., pulse 190, respiration 40. The cat was still active but much less so than before. At 4.30 p.m. the temperature was 40.9°C.; the condition of the cat was much the same as at 3.00 p.m., except that the intervals of quiet were longer.

The effect of anesthesia with ether on the symptoms and hyperthermia produced by morphine was illustrated in the following experiment, and it was shown that a light narcosis was sufficient to suppress both. When the action of the ether was allowed to wear off, the rectal temperature rose and the ordinary symptoms developed.

Cat 628; female; weight 2.61 kgm. The left superior cervical ganglion had been excised 29 days previously, when the cat weighed 2.10 kgm. At 8.45 a.m. the rectal temperature was 38.4°C., pulse 148, respiration 45. The cat was now anesthetised, first with a little chloroform and then with ether. The left pupil, which had been narrower, became wider than the right. At 9.15 a.m., temperature 37.7°C., pulse 224, respiration 30. Room temperature 25.5°C. At 9.20 a.m., 30 mgm. morphine; discontinued ether at 9.30 a.m. At 9.45 a.m., left pupil was maximal, and larger than the right; left nictitating retracted, right protruded; reflexes not exaggerated; temperature 37.55°C. pulse 188, respiration 13.

10.00 a.m. Temperature 37.65°, pulse 192, respiration 20, pupils same as before, but right nictitating is also retracted. Reflexes slightly exaggerated. Enough ether was given from 10.10 a.m. to 10.20 a.m. to keep the animal lightly narcotised, and reflex excitability (eyes and legs) disappeared. No more ether thereafter.

10.30 a.m. Temperature 38.4°C., pulse 228, respiration 18.

11.00 a.m. Left pupil maximal and larger than right, reflexes obtainable. Temperature 37.5°C., pulse 204, respiration 18.

11.30 a.m. Pupils about equal ($\frac{3}{4}$ to $\frac{7}{8}$ maximal), nictitating membranes retracted. Temperature 38.55°C. pulse 240, respiration 22. Cat begins to get restless and to "paw" around.

12.00 m. More active. Temperature 40.2°C., pulse 172, respiration 27.

1.00 p.m. Active. Temperature 41.0°C., pulse 168, respiration 25. Pupils equal. Same condition at 2.00 p.m. (temperature 40.8°C.. pulse 167, respiration 26).

3.00 p.m. Less active. Temperature 40.17°C. Pupils equal ($\frac{2}{3}$ maximal). Animal returned to stock.

We do not propose to discuss here the mechanism of morphine hyperthermia in cats. This could not be done to advantage in the absence of calorimetric observations (direct or indirect). Boeck and Bauer (3) found the carbon dioxide excretion increased about 40 per cent in the one cat observed. The oxygen intake was increased on the average only 13 per cent; they make no mention of any temperature measurements.   It is commonly admitted that increased heat production can exist without hyperthermia, and hyperthermia in the absence of increased heat production. As far as can be judged from the greatly increased respiratory rate, the red nose and tongue, the warm sweating pads of the feet, and the warmth and hyperemia of the skin, especially obvious over previously shaved areas, the heat loss is increased by morphine in cats at the same time as the rectal temperature.   This would imply a great increase in the heat production.

It is well known that in dogs and rabbits, morphine does not cause an increase but rather a decrease of body temperature. This is usually most conspicuous when the animal is stretched out on a board, but it is also seen when it is free, as was the case with the rabbits in our experiments.   In a dog under morphine but not unconscious, and tied on a board the following rectal temperatures were observed: 38.1° (12.15 p.m.), 38.0° (12.30 p.m.), 36.9° (1.00 p.m.), 36.5° (1.30 p.m.), 36.1° (2.15 p.m.), 36.0° (2.45 p.m.).

The rise of temperature produced by morphine in cats may be in some measure associated with the increased muscular movement. But as already pointed out, this cannot be the sole and

may not be the main factor.   For the amount of muscular contraction, even when the restlessness is extreme, is not to be compared with that elicited in the revolving cage, and yet the morphine rise of temperature is usually greater.   Further, in the morphine experiments the increase of temperature is by no means always greatest in the animals which show the greatest degree of excitement and of muscular movement.   This does not of course imply that the morphine hyperthermia can be obtained when the skeletal muscles are eliminated, as by curara.   Nor does the prevention of the rise of temperature by ether imply that the hyperthermia is a "nervous fever" in the sense in which some writers have employed the term in connection with cocain hyperthermia.   U. Mosso (4) alleged that the so-called cocain fever can be elicited after section of the spinal bulb and after curara. In one experiment on a dog, curarised so as to be motionless, a rise of rectal temperature of 4.7°C. was seen in forty-five minutes. He also obtained a substantial rise of temperature with strychnine after complete curarisation.

Richet and Langlois, and Reichert (5) were unable to observe any increase of temperature in animals paralysed by curara, and that was also the experience of one of us, as illustrated in figure 1. The rectal temperature, the temperature shown by a thermometer fastened permanently in a pouch of skin, and the air temperature in a still room were read at intervals, and curves plotted.   The animal was a medium sized dog.   The air for artificial respiration was warmed and saturated with watery vapor by passing through a large water valve kept at 35°C.   The loss of heat was therefore entirely from the skin.   The other details are given in the figure. It will be seen that the superficial and rectal temperatures both run nearly parallel with the air temperature after the administration of curara, unaffected by subsequent injection of cocain. A little ether and chloroform mixture was given occasionally before curarisation and also, thereafter, but none for a 'ong time after administration of cocain.   Curarisation caused no obvious chan e in the temperature curves.   When artificial respiration was stopped at the end of the experiment and the heart beat ceased, the rectal and skin temperatures fell at first approximately

parallel with each other, but the superficial temperature necessarily somewhat faster. For a short time it can be assumed that the rate of loss of heat from the surface, measured by the rate of cooling, is about the same as before death. Stoppage of the arti-
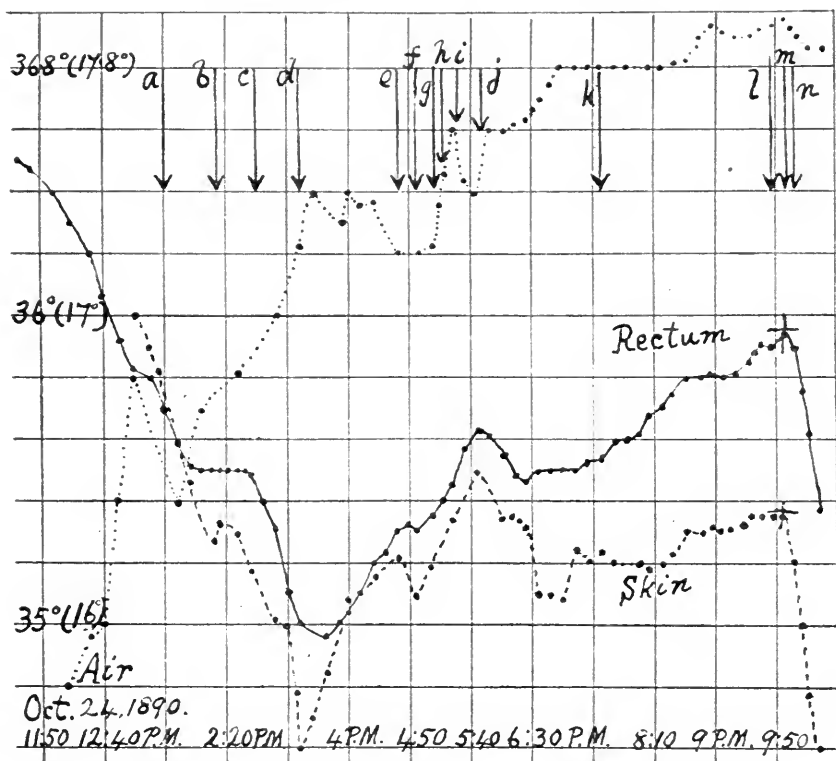

FIG. 1. TEMPERATURE CURVES (DOG)

Continuous curve, rectal temperature; interrupted curve, temperature in a fold of skin; dotted curve, room temperature. Temperatures plotted as ordinates. The numbers in parentheses are for the skin temperature. At *a*, artificial respiration was begun; *b*, subcutaneous injection of 2 cc. of an active 1 per cent curara solution; *c* and *d*, repetitions of this dose. Ether and chloroform mixture stopped at *d*, recommenced at *e*; stopped at *f*; given again at *g*; stopped at *h*. Animal under curara but not deeply. At *i* injected subcutaneously 60 mgm. cocain hydrochloride, at *j* 60 mgm., at *k* 60 mgm. At *l* gave ether and chloroform in large amount for 4 minutes to kill the animal; at *m* (marked by a cross on the temperature curves) stopped artificial respiration. At *n* the heart beat was no longer felt. One of the squares represents 0.2°C., and fifty minutes of time. The changes of temperature are therefore greatly exaggerated.

ficial respiration would not affect this, as practically no heat was being given off by the lungs.

In one cat (599) the influence of cold in modifying the morphine hyperthermia was observed. The symptoms developed as usual, but the maximum rise of temperature was diminished and delayed.

Cat 599; female (pregnant); weight 2.5 kgm.

April 26, at 10.50 a.m. the temperature was 38.3°C., pulse 186, respiration 66. Morphine (20 mgm.) was injected, and the cat placed in a refrigerator with a glass door (at 5° to 6°C., rising to 8°C. at end of experiment). At 10.55 a.m. moderate salivation, pupils dilated. At 11.15 a.m., it was becoming restless. At 11.30 a.m. temperature 39.05°C.; more restless. At 12.05 p.m., temperature 39.4°C.; cat very restless, running around, etc. At 12.45 p.m. and 1.30 p.m., the temperature was 39.55°C. and 39.3°C. respectively, and the animal was very restless and moving around.

At 1.40 p.m. the cat was removed from the refrigerator, dried and placed in a cage at room temperature (21.7°C.). At 2.15 p.m. the temperature was 40.0°C. The animal was getting more active, running around, pawing etc. The restlessness seemed in part playful. At 3.00 p.m. the temperature was 40.5°C.; at 4.00 p.m. 40.15°C. (still very active); at 5.30 p.m. 39.55°C. (still active, but less so than at last observation. Purred while the observation was being made). Next morning at 9.00 a.m. temperature was 37.9°C.; the animal appeared normal and took a little milk.

April 29. Weight 2.38 kgm. Only water, but no food except the milk on the morning of April 27, had been given since evening of April 25. The cat was not put into the refrigerator in this experiment.

At 10.00 a.m. Temperature 38.1°C.; room 20.5°C.

10.30 a.m. Blood sugar (femoral vein) 0.136 per cent.

10.35 a.m. Injected 30 mgm. morphine. At 10.50 a.m. pupils dilated; the cat is pawing (apparently playfully) and getting active. At 11.15 a.m. it is moderately active.

11.30 a.m. Temperature 40.3°C.; blood sugar 0.135 per cent.

1.05 p.m. Temperature 40.6°C.; room 21.1°C., symptoms the same as at 11.15 a.m. Blood sugar 0.184 per cent.

1.16 p.m. to 1.36 p.m. Asphyxia (intermittent, with towel). Blood sugar 0.228 per cent; temperature at 1.40 p.m. 40.5°C. Glycogen in the liver 0.19 per cent. The animal was found to be pregnant (more than half term).

In this experiment, although the conditions were such as to deplete the glycogen store (deprivation of food, and the previous morphine experiment), the maximum rise of temperature was 2.5°C. Some increase of blood sugar was also caused by the morphine, and a definite hyperglycemia by asphyxia. It cannot be known how much glycogen was present in the liver at the beginning of the experiment; it probably can be assumed that it was several times as great as the amount found at the end. The liver weighed 60 gm., and allowing only for the raising of the blood sugar percentage to that found after asphyxia, three times as much glycogen must have been contained in the liver as was found, if the sugar came from liver glycogen. This takes no account of sugar derived from glycogen which may have been burnt, nor on the other hand of the possibility that some of the sugar might have been derived from the glycogen reserves of the embryos or placenta.

MORPHINE HYPERTHERMIA AND HYPERGLYCEMIA IN NORMAL CATS

In all the remaining experiments the blood sugar content was estimated from time to time by Pearce's autoclave modification of Benedict's method. As in all our previous work with this method, a supersaturated solution of picric acid was employed (the solution being saturated hot, then allowed to cool to room temperature and used before crystals have separated). Parallel observations on the rectal temperature were made, and the pulse and respiratory rates noted. The glycogen content of the liver was estimated by Pflüger's method at the end of the experiment, the liver being removed immediately after killing the animal by a blow on the head.

When there was no hyperglycemia in the normal cats after morphine the content of glycogen in the liver was always low. But the converse did not hold, that is to say, a good hyperglycemia was sometimes found when the glycogen content of the liver at the end was small. In these cases, as already pointed out, it seems fair to assume that at the beginning of the experiment there may have been considerably more glycogen in the liver. Illustrative experiments are cited (on cats 613, 603 and 609).

In cat 613, although specially fed for 12 days (with a stew of liver, potatoes and rice, to which milk was added after it was cooked, and sometimes dextrose), the liver at the end of the experiment contained only a trace of glycogen (0.037 per cent). The animal had eaten well, but its head and legs were mangy, and its rectal temperature was above normal before morphine was given.

Cat 613; female; weight 2.53 kgm.

10.40 a.m. Rectum 39.45°C., room 23.3° to 24.5°C.; the animal was excited; cried and fought all the time. Blood sugar 0.086 per cent.

10.45 a.m., 5 mgm. morphine. At 10.55 a.m., it defecated and micturated. Rectum 39.2°C., (11.15 a.m.), 39.6° (11.45 a.m.), 40.3° (12.15 p.m.). The cat appears moderately uneasy, playful at times.

1.00 p.m. Restless and apparently sometimes playful; cries and purrs alternately. Rectum 40.35°C.; blood sugar 0.094 per cent.

1.45 p.m. Condition unchanged; pupils wide. Rectum 40.55°C.; 40.45°C. (2.15 p.m.); 40.55°C. (2.45 p.m.). Condition about the same.

3.30 p.m. Not so active. ' Rectum 40.1°C. blood sugar 0.080 per cent.

4.10 p.m. Quieter. Rectum 40.3°C.; 40.0°C., (5.00 p.m.).

5.35 p.m. Quiet. Rectum 39.55°C.; blood sugar 0.075 per cent. After 20 minutes asphyxia, blood sugar 0.107 per cent. Total weight of liver 78.2 gm. Glycogen in liver 0.037 per cent.

It might be thought that the relatively small dose of morphine in cat 613 was responsible for the negative result on the blood sugar. But the fact that asphyxia, which is one of the surest methods of causing hyperglycemia when a sufficient glycogen store exists, also gave a negative result, suggests that this is not the explanation. This ia good illustration of the fact that the rise of internal temperature is not at all closely dependent upon an increase in the blood sugar content. When a definite hyperglycemia develops after administration of morphine, there is of course a rough parallelism between the blood sugar and the temperature curves. But this has no great significance. In rabbits and in dogs where there is no hyperthermia, morphine causes hyperglycemia.

Cat 603; female; weight 3.325 kgm. (in early pregnancy). The animal was selected from the stock, as well nourished, and was fed on the special diet (with in addition, raw liver) for 4 to 5 days. It was an old cat (toothless) and somewhat mangy around the ears.

9.30 a.m. Rectum 38.5°C.; pulse 188, respiration 34. Blood sugar (9.45 a.m.) 0.156 per cent.

9.50 a.m. Morphine (40 mgm.). At 10.00 a.m. the pupils were dilated and some signs of uneasiness. At 10.55 a.m. the animal was very restless, running around the cage. Hairs erect. Respiration 32 (at rest), 48 to 54 after a restless spell. Pain sensation dulled.

11.20 a.m. Extremely restless and apprehensive; pupils not quite maximal. Room 20°C.; rectum 41.4°C.; pulse 200 (bounding); respiration about 80. Blood sugar (11.30 a.m.) 0.375 per cent.

12.30 p.m. Respiration becoming panting, going up to 140 to 160 a minute during the restless spells and to 60 to 80 (deeper) during intervals of quiet, which are becoming shorter. Pupils $\frac{3}{4}$ to $\frac{7}{8}$ maximal. At 12.38 p.m. there was a strychnine-like convulsion lasting 30 to 45 seconds, followed by relaxation, the animal lying in the cage and panting (respiration over 300 a minute). Pupils maximally dilated during the convulsion, then returning to former width.

12.50 p.m. Tongue and mucous membrane of mouth red. Rectum 42.5°C. This was the maximum (4°C. above the initial temperature), pulse 260 to 270. There were two more convulsions when it was attempted to get blood, in the last of which the animal died. Blood was at once obtained from the heart (at 1.10 p.m.); it contained 0.37 per cent of dextrose. Weight of liver 97.2 gm., glycogen content of liver 0.30 per cent.

Even to permit the increased blood sugar content reached, without allowing anything for combustion, the liver must have contained 3 times as much glycogen at the beginning as at the end of the experiment, on the assumption that the sugar all came from the liver glycogen. It seems probable that there was originally a fair glycogen content.

Cat 609, female, weight 2.52 kgm. Fed for five days like cat 603.

8.50 a.m. Rectum 38.3°C.; room 21° to 21.5° C.; pulse 200; respiration 38. Blood sugar (9.00 a.m.) 0.13 per cent.

9.02 a.m. Morphine (20 mgm.). Rectum 38.1°C. (9.18 a.m.: no symptoms, but pupils somewhat wider); 38.45°C.. (9.30 a.m.) (quiet

but getting apprehensive); 38.7°C., (9.45 a.m.) (crying at times, hairs erect, pupils ¾ maximal); 39.3°C. (10.00 a.m.): 39.55°C. (10.15 a.m.); 40.0°C. (10.30 a.m.) (at times restless; reflexes markedly exaggerated); 40.2°C. (10.45 a.m.); 40.6°C. (11.00 a.m.) (more active; pulse 186, respiration 34 to 36 when quiet, becoming quicker like the pulse during activity).

11.05 a.m.   Blood sugar 0.26 per cent.   Rectum 40.9°C. (11.30 a.m.) (quite active at times, more so when approached; pupils almost maximal); 41.1°C. (12.00 m).; 41.1°C. (12.45 p.m.) (symptoms unchanged).

1.30 p.m.   Quite active at times, but spells of quiet are longer; respiration 34 (quiet), 66 (excited); rectum 40.6°C.   Blood sugar (1.35 p.m.) 0.17 per cent.

2.10 p.m.   Less active.   Rectum 40.3°C.; pulse 184, respiration 56.

2.50 p.m.   Quieter.   Rectum 40.3°C.   Blood sugar (2.55 p.m.) 0.15 per cent.   After twenty-five minutes intermittent asphyxia, blood sugar 0.18 per cent.   Rectum 39.8° (at 3.20 p.m., during the period of intermittent asphyxia).   Weight of liver 68.1 gm., content of glycogen 3.66 per cent.

In two other normal cats (608 and 614), which both showed a fair hepatic glycogen store at the end of the experiment, the results were much the same as in cat 609, although, with a smaller dose of morphine, the increase of internal temperature was not so pronounced.

Cat 608; female; weight 3.06 kgm.   Specially fed as in 603 for four days.

9.20 a.m.   Rectum 38.6°C.   The animal was much excited and fought angrily while the temperature was being taken and the first blood specimen collected.   Blood sugar 0.09 per cent.

9.30 a.m.   Morphine (10 mgm.).   Rectum 39.5°C. (10.00 a.m.) (only slightly apprehensive, hair erect, pupils ¾ to ⅞ maximal); room 19° to 21°C.

10.30 a.m.   Active when approached.   Pulse 175, respiration 47; rectum 40.4°C.

11.00 a.m.   Cat purring, but tries to scratch.   Pulse 240, respiration 44.   Rectum 41.05°C.   Blood sugar 0.15 per cent.

11.35 a.m.   Rectum 40.7°C.; 40.65°C. (12.15 p.m.), 40.8°C. (12.50 p.m.).   Condition the same.

1.25 p.m.   Rectum 40.65°C.   Blood sugar (1.30 p.m.) 0.18 per cent.

2.05 p.m. Not so restless. Rectum 40.3°C; 40.3°C. (2.35 p.m.);
40.25°C. (3.05 p.m.); 40.25°C. (3.35 p.m.).

4.05 p.m. Rectum 40.25°C.; room 22.8°C.: pulse 198, respiration 40.
Blood sugar (4.10 p.m.) 0.10 per cent. Intermittent asphyxia for
25 minutes was now started. Blood sugar after asphyxia 0.14 per
cent. Rectum 39.45°C. The liver weighed 93.2 gm. and contained
3.11 per cent of glycogen.

It may be pointed out here that two of the animals (613 and
608) which were most excited, before and during the collection of
the first blood specimen, showed about the smallest percentages of
blood sugar (0.08 and 0.09).

Cat 614; male, weight 3.95 kgm. The cat had no special feeding, as
it had only been a day or two in the laboratory, but received by stomach
tube 25 cc. of 5 per cent cane sugar the day before the experiment.

8.30 a.m. Rectum 39.3°C., pulse 196, respiration 34; room 22.8° to
24.5°C. Blood sugar (8.40 a.m.) 0.12 per cent.

8.45 a.m. Morphine (10 mgm.). At 9.15 a.m. no symptoms.
Pulse 160, respiration 26. Rectum 39.45°C.; 40.0°C. (9.45 a.m.),
40.0°C. (10.15 a.m.), 40.15°C. (10.45 a.m.). The cat had defecated,
was apprehensive but not active. Pupils ¾ maximal.

11.15 a.m. Condition much the same; reflexes increased, especially
to sound. Pulse 160, respiration 28. Rectum 40.3°C. Blood sugar
(11.20 a.m.) 0.22 per cent.

11.45 a.m. Rectum 40.1°C.; 40.0°C. (12.15 p.m.), 39.95°C. (1.00
p.m.) (quieter).

1.30 p.m. Rectum 40.0°C. Blood sugar (1.35 p.m.) 0.12 per cent

2.00 p.m. Condition the same. Quiet. Rectum 40.2°C.; 40.15°C.
(2.30 p.m.); 40.0°C. (3.00 p.m.).

3.30 p.m. Quiet, occasionally pawing. Seems dazed. Rectum
40.1°C. Blood sugar (3.35 p.m.) 0.13 per cent. At 3.55 p.m. after
five minutes intermittent asphyxia, blood sugar 0.20 per cent.

The liver weighed 124.5 grams and contained 2.5 per cent of glycogen.

In some of the animals although a definite hyperglycemia was
caused by the morphine, the glycogen store of the liver was found
quite small at the end, and the blood sugar content tended to fall
in the later specimens even below the value obtained before
administration of the drug. The effect of asphyxia was then

negative. Our provisional interpretation of this result is that the liver initially contained a fair stock of glycogen, which became exhausted in the course of the experiment with a tendency to hypoglycemia, so that when asphyxia was induced at the end there was not enough glycogen left to permit of an increase in the blood sugar. An example is given (cat 610).

Cat 610; female; weight 2.2 kgm. No special feeding.

8.30 a.m. Rectum 37.4°C.; room 21°C.; pulse 226, respiration 24. Blood sugar (8.55 a.m.) 0.14 per cent.

8.58 a.m. Morphine (15 mgm.). Rectum 37.3°C. (9.00 a.m.).

9.10 a.m. Reaction beginning; occasional cry; pupils wider; rubbing head and back on floor. Rectum 37.9° (9.30 a.m.) (more movement, playful, pupils wider); 38.8° (10.00 a.m.) (quite active, not ugly).

10.30 a.m. Rectum 40.15°C.; pulse 262, respiration 32. Blood sugar (10.40 a.m.) 0.20 per cent.

11.15 a.m. Active and apprehensive, but quieter at times. Rectum 40.8°C.

12.00 m. Symptoms the same. Rectum 41.7°C.; pulse over 300 and full, respiration 60 to 80 when active. Purrs when hand put on chest. Blood sugar (12.05 p.m.) 0.23 per cent.

12.50 p.m. A little quieter; pulse and respiration as at last observation. Rectum 41.1°C.; 41.1°C. (1.20 p.m.); 41.0°C. (1.50 p.m.).

2.30 p.m. Quieter; pulse rapid and not so strong as before, respiration shallower and slower. Pupils ¾ maximal. Rectum 40.6°C. Blood sugar (2.40 p.m.) 0.14 per cent.

3.15 p.m. Somewhat quieter. Rectum 39.8°C.

3.50 p.m. Quiet; pulse rapid; respiration 30. Rectum 39.8°C. Blood sugar (4.00 p.m.) 0.11 per cent.

4.30 p.m. Seems weak and stupid; pulse 280 to 300 (weaker), respiration 31 (shallow when quiet, deeper when moving). Rectum 39.1°; 38.8° (5.00 p.m.). Blood sugar (5.05 p.m.) 0.12 per cent. After 15 minutes asphyxia blood sugar (5.22 p.m.) 0.11 per cent. The liver weighed 92.5 gm. and contained 0.21 per cent of glycogen.

In all the experiments hitherto cited the morphine was administered hypodermically. The following (cat 618) is an example of an experiment with intravenous injection. The general results were the same, but the symptoms and the rise of rectal temperature occurred earlier (2.5°C. in 15 minutes). The moderate hypergly-

cemia was replaced in the later observations by even a smaller percentage of sugar than before the morphine was given, and the hepatic glycogen content was very low, as if the glycogen had been rapidly swept out. It is, however, impossible to know whether at the beginning the liver was fairly well filled or not.

Cat 618; female (pregnant); weight 2.97 kgm. No special feeding.

9.30 a.m. Rectum 38.4°C.; room 24°C. Pulse 190, respiration 52 to 56. Blood sugar (9.35 a.m.) 0.11 per cent.

9.45 a.m. Morphine (15 mgm.) intravenously. Pulse 144 (9.46) bounding; respiration 56. The cat became active as soon as it was placed in the cage.

10.00 a.m. Pulse 180 (full), respiration 90, pupils dilated. Animal very active. Rectum 40.9°C. Reflexes increased. No salivation. Pads of feet dry. Blood sugar (10.05 a.m.) 0.16 per cent. (A control showed that the presence of morphine in the blood, as such, did not influence the sugar estimations.)

10.40 a.m. Very active. Rectum 41.5°C.; 41.7°C. (11.00 a.m.), pulse 160 (bounding), respiration rapid and panting.

11.30 a.m. Pulse and respiration the same, pupils maximal. Rectum 41.65°C. Blood sugar (11.35 a.m.) 0.17 per cent.

12.00 m. No change. Rectum 41.6°C.; 40.9°C. (1.00 p.m.).

2.00 p.m. Condition the same, very active. Rectum 40.7°C. Pupils maximal. Blood sugar (2.05 p.m.) 0.08 per cent.

3.00 p.m. A little less active. Rectum 40.3°C.; 40.4°C. (3.30 p.m.), quieter; pulse over 200 (full), respiration 38 to 40. Blood sugar (3.35 p.m.) 0.08 per cent. After asphyxia for 25 minutes, blood sugar (4.05 p.m.) 0.075 per cent. The liver weighed 59 gm. and contained 0.015 per cent of glycogen.

One cat out of about 30 employed in the investigation showed the characteristic symptoms after morphine only slightly. There was no hyperglycemia, the blood sugar declining rather than rising, and the rectal temperature fell. The animal had been found in a starving condition and brought to the laboratory and was used without having received any food except a little milk 2 days before the experiment. It was considerably emaciated.

Cat 602; male; weight 2.20 kgm.

9.00 a.m. Room 17°C. Rectum 37.2°C.; struggling brought it up to 37.6°C. Pulse 218, respiration 36. Blood sugar (9.30 a.m.) 0.13 per cent.

9.40 a.m. Morphine (30 mgm.) At 9.50 a.m. began to show signs of apprehension, and these increased somewhat till 11.00 a.m., when the hair of the tail was erect and the pupils $\frac{3}{4}$ to $\frac{7}{8}$ maximal. The animal remained sitting quietly in the cage, and showed no distinct exaggeration of movement at any time.

11.00 a.m. Rectum 35.8°C. Blood sugar 0.093 per cent.

12.30 p.m. Condition unchanged. Pulse 216, respiration 48. Rectum 35.1°C.; room 21°C.

1.00 p.m. Blood sugar 0.092 per cent. After twenty-five minutes intermittent asphyxia blood sugar (at 1.41 p.m.) 0.010 per cent. The liver weighed 70 gm. and contained 0.22 per cent of glycogen.

From the whole series of experiments on normal cats it may be concluded that hyperthermia is a practically constant effect of morphine and obtained in animals with a very small store of hepatic glycogen, just as in animals whose livers are well filled with glycogen. There is no close relationship between the hyperthermia and the hyperglycemia. This is in accordance with the well known fact that in dogs and rabbits morphine causes hyperglycemia although the body temperature is reduced rather than increased. One experiment on a normal rabbit, made in the same way as on the cats, may be quoted.

Rabbit 637; male; weight 2.295 kgm. Specially fed with carrots daily and about 1 gram of dextrose daily in the water, in addition to the usual diet (oats and hay), for four days.

9.00 a.m. Rectum 39.1°C. Pulse and respiration too rapid to count. Blood sugar (ear vein) 0.12 per cent.

9.10 a.m. Morphine (50 mgm.).

9.30 a.m. Pulse and respiration still very rapid; getting sleepy. Rectum 39.25°C. At 10.00 a.m. rectum 39.45°C., pulse 178, respiration 50, pupils $\frac{2}{3}$ maximal.

10.40 a.m. Sleeping. Rectum 39.1°C. Pulse, respiration and pupils the same. Blood sugar 0.26 per cent.

11.30 a.m. Rectum 39.0°C., pulse 200, respiration 40.

12.35 p.m. Rectum 38.4°C., pulse 225, respiration 36, pupils nearly ¾ maximal. Animal quiet. Blood sugar 0.22 per cent.

1.30 p.m. Rectum 38.0°C., pulse 250, respiration 44.

2.20 p.m. Rectum 37.9°C., pulse over 300, respiration 35. Blood sugar (2.40 p.m.) 0.15 per cent. After twenty-five minutes intermittent asphyxia, blood sugar (3.15 p.m.), 0.21 per cent. The liver weighed 70.8 gm. and contained 3.34 per cent of glycogen.

MORPHINE HYPERTHERMIA IN CATS AFTER REMOVAL OF THE GREATER PORTION OF THE ADRENALS AND DENERVATION OF THE REMAINING FRAGMENT

At the beginning of the paper two experiments were mentioned in which it was shown that removal of five-sixths of the total adrenal tissue did not modify the general symptoms or the hyperthermia induced by morphine. In the experiments now to be described, in addition to excision of the major part of the adrenal mass, the remaining fragment was denervated, so that if any medullary tissue was left in the stump its output of epinephrin would be interfered with. The result was the same as before. The general behavior of the animals after the morphine and the rise of body temperature followed the same course in the operated as in the normal animals. As regards the blood sugar, which was also estimated in this series, it did seem, however, as if a difference emerged, the morphine hyperglycemia being apparently more readily elicited in the normal animals. If this is the case, it would constitute the first real distinction observed by us. We have already shown that other forms of experimental hyperglycemia (6), (ether, asphyxia, piqûre) are readily obtained in rabbits which have survived double adrenalectomy, and in cats deprived of one adrenal and with the other denervated, provided that the animal has been placed under conditions allowing a good store of glycogen to accumulate in the liver. In the present investigation this has been repeatedly confirmed for asphyxia in cats with only a fragment of adrenal tissue left, and that denervated. After these adrenal operations, however, a sufficient interval must be allowed to elapse in order to ensure an adequate accumulation of glycogen, and a negative result when an esti-

mation of the liver glycogen shows that the store was deficient, has, of course, no value.   In a number of our partially adrenalectomised cats this was the case, and further experiments are in progress on this subject.   In three of the cats (611, 612, and 615) the glycogen content was so low that a hyperglycemia could not be expected, and as a matter of fact no definite hyperglycemia was caused by asphyxia, perhaps the most constant method of producing one.

Cat 611; male; 2.25 kgm.   January 10.   Excised ⅗ of right adrenal and on January 28 all the left adrenal.   On February 10 it was in excellent condition and weighed 2.725 kgm.   It was used (as cat 499) for experiments on exercise (see preceding paper) (1), the last time on February 23.   On April 27 it weighed 3.17 kgm. and was in excellent health.   On that date the remaining fragment of the right adrenal was denervated, the major and minor splanchnics being cut and most of the right semilunar ganglion excised.

May 16.   Weight 3.16 kgm.   It had been on the special diet for a week.   As in all the animals recently operated on, the hair had not grown much over the shaved area (about ⅓ of the total surface). Room 18° to 21°C.

9.10 a.m.   Rectum 37.8°C.   Pulse 176, respiration 48.   Blood sugar 0.12 per cent.

9.20 a.m.   Morphine (20 mgm.).   9.45 a.m.   Pulse and respiration about the same; pupils slightly wider.   Occasionally cries.   Rectum 37.95°C.   At 9.50 a.m. beginning signs of uneasiness.   At 10.15 a.m. moderately active, pupils nearly maximal.   Rectum 38.45°C.

10.45 a.m. more active, hairs erect, reflexes exaggerated.   Rectum 39.45°C.; 40.0°C. (11.15 a.m.).

11.45 a.m.   Condition the same.   Rectum 40.8°C.   Blood sugar (11.55 a.m.) 0.13 per cent.

12.30 p.m.   Apprehensive, pupils nearly maximal, fought and scratched.   Rectum 40.85°C.; 40.4°C. (1.00 p.m.).

2.00 p.m.   A little quieter.   Rectum 40.0°C.   Blood sugar 0.14 per cent.

2.30 p.m.   Quieter.   Rectum 39.4°C.; 39.5°C. (3.00 p.m.); 39.8°C. (3.30 p.m.).

4.00 p.m.   Quiet.   Pulse 276, respiration 34, rectum 40.05°C.   Blood sugar (4.10 p.m.) 0.16 per cent.

4.40 p.m. A little more active again. Rectum 40.2°C.; 39.8° (5.25 p.m.). Blood sugar (5.30 p.m.) 0.15 per cent. Blood sugar after twenty-five minutes intermittent asphyxia (6.00 p.m.) 0.16 per cent.

The liver weighed 75.1 grams and contained 0.04 per cent of glycogen. The adrenal fragment weighed 0.16 gm.

Cat 612; female; weight 2.19 kgm. January 10. Excised $\frac{1}{3}$ to $\frac{2}{3}$ of left adrenal, and on February 2 all of the right gland. The animal recovered well and was used for exercise experiments (as cat 501), for the last time on March 3. On March 8 it weighed 2.33 kgm.

April 25. Denervated remnant of left adrenal, which apparently had hypertrophied somewhat.

May 17. Weight 2.78 kgm. Had special diet for eight days. Fully one-third of the skin (over operative field) was not well covered with hair. Room 22° to 23° to 21°C.

8.45 a.m. Rectum 37.75°C. Pulse 210, repiration 28. Animal quiet. Blood sugar 0.13 per cent.

8.55 a.m. Morphine (25 mgm.). The symptoms were slight at 9.15 a.m. At 9.30 a.m. the animal was quiet but apprehensive; rectum 37.75°C. At 10.00 a.m., hairs erect, reflexes exaggerated, pupils somewhat wider; rectum 38.8°C. At 10.30 a.m., active at times; playful and purring. Rectum 40.15°C.

11.00 a.m. More active. Pads red and very moist. Rectum 41.3°C. Blood sugar (11.10 a.m.) 0.13 per cent

11.30 a.m. Condition about the same, not very active. Rectum 41.8°C.; 41.6°C. (12.00 m.); 41.95°C. (12.30 p.m.)

1.00 p.m. Condition the same. Rectum 41.8°C. Blood sugar 0.14 per cent.

1.30 p.m. Condition the same. Pulse very rapid, respiration 75. Rectum 41.3°C.; 41.2°C. (2.00 p.m.); 41.1°C. (2.30 p.m.) (not quite so active).

3.15 p.m. Rectum 41.3°C. Blood sugar 0.13 per cent.

4.00 p.m. Ears and skin red. Pulse strong and very rapid; respiration 36; pupils less wide. Rectum 40.6°C.; 40.5°C. (4.30 p.m.).

5.00 p.m. No change. Rectum 40.55°C. Blood sugar 0.12 per cent. At 5.35 p.m., after 25 minutes asphyxia, blood sugar 0.12 per cent.

The liver weighed 63.6 grams and contained 0.56 per cent of glycogen. The fragment of adrenal weighed 0.083 gm.

Cat 615; female; weight 2.33 kgm.

January 18.   Excised more than $\frac{1}{10}$ of right adrenal, tying the artery. On February 3 excised $\frac{2}{3}$ to $\frac{3}{4}$ of the left adrenal.   The animal was used for exercise experiments (as cat 505), for the last time on April 19.   On May. 6 it was in excellent condition and weighed 2.735 kgm. The remnant of the left adrenal was denervated, the left major and minor splanchnics being cut, and the greater part of the left semilunar ganglion excised.

May 25.   Weight 2.7 kgm.   Special diet (the stew of liver, rice, potatoes and milk already described) since May 6, in addition to raw liver and milk.   Also 75. cc. of 5 per cent cane sugar by stomach tube on May 23 and 24.

8.40 a.m.   Rectum 39.6°C., room 22.7°C.   Pulse 136, respiration 28.   Blood sugar 0.09 per cent.

8.50 a.m.   Morphine (25 mgm.).   At 9.15 a.m.   no symptoms, except somewhat wider pupils.   Rectum 39.2°C.; 39.2°C. (9.45 a.m.) (slightly apprehensive, reflexes increased, pupils $\frac{7}{8}$ maximal); 39.45°C. (10.15 a.m.) (quiet but very apprehensive, pulse 210, respiration 36).

10.45   a.m.   Condition   the   same.   Pupils   maximal.   Rectum 39.95°C.   Blood sugar 0.11 per cent.   At 11.15 a.m., rectum 40.8°C. Active at times.

11.45 a.m.   Rectum 41.2°C.   Blood sugar 0.11 per cent.

12.15 p.m.   Rectum 41.7°; 41.9°C. (1.00 p.m.).   Condition the same. Pilo-motor effects when active.

1.30 p.m.   Rectum 42.0°C.   Blood sugar (1.40 p.m.) 0.10 per cent.

2.15   p.m.   Rectum   41.55°C.;   41.7°C.   (2.45   p.m.),   condition unchanged

3.30 p.m.   Rectum 41.45°C.   Pulse 250 to 280, respiration 30. Blood sugar 0.10 per cent.   After twenty-five minutes asphyxia blood sugar (at 4.05 p.m.) 0.09 per cent.

The liver weighed 55.1 grams and contained only a trace, not more than 0.02 per cent of glycogen.

It is again pointed out that if the morphine hyperglycemia is as dependent upon a good glycogen store as the other hyperglycemias previously studied, it would have been impossible to expect a definite hyperglycemia in these three animals.   The interval since the last operation (about three weeks) was apparently too short to permit much glycogen to accumulate.   This accords with our

previous experience (6), although it was shown then, and is again shown in other experiments in this paper, that when a sufficient interval is allowed, animals after these adrenal operations lay down large quantities of glycogen under appropriate conditions.   In the next experiment an attempt was made to ascertain beforehand whether the conditions necessary for eliciting hyperglycemia existed, by subjecting the cat to a period of partial asphyxia. The result having been positive as regards the blood sugar, the animal was allowed a period of eight days on the special diet to recuperate its glycogen store, and an experiment with morphine was then made.   The animal, however, had a convulsion when only one blood specimen had been collected after morphine, and the final specimen was collected under conditions which rendered it impossible to be certain whether the apparent small increase in sugar was associated with the morphine action or with asphyxia.   It is impossible that in any case the experiment could have yielded a definite result since the liver was found to contain only a trace of glycogen, the interval since the previous asphyxia experiment being presumably too short for recuperation.

Cat 629; female (pregnant); weight 2.56 kgm.

June 3.   Excised the right adrenal and $\frac{3}{5}$ of left, and denervated the fragment.   On June 20 she had a litter of immature embryos. On July 5, the weight was only 1.77 kgm., although the diet was "special" for ten days.

July 5, 9.45 a.m.   Blood sugar 0.09 per cent.

10.10 a.m.   After twenty minutes intermittent asphyxia, blood sugar 0.21 per cent.

July 13.   Special diet still continued. Weight 1.85 kgm. At 8.20 a.m., rectum 38.6°C.; room 25.5° to 26.5°C.   Pulse 152, respiration 34. Blood sugar 0.08 per cent.

8.30 a.m.   Morphine (30 mgm.) subcutaneously.   At 8.35 a.m. the cat was quite active and showing distinct effects of the morphine. This was never observed with hypodermic injection after so short an interval in any other cat.   As the injection was made into the site of one of the previous incisions, it is possible that veins might have been directly punctured, or absorption may have been more rapid than usual.

8.45 a.m.   Rectum 39.7°C.   Pulse 200, respiration 34; cat very active.

9.00 a.m.   Rectum 40.9°C.   Pulse 220 (forcible), respiration 34. Blood sugar 0.09 per cent.

9.50 a.m.   Rectum 41.3°C.   Pulse too rapid to count, respiration 48.; hair up on back and tail, pupils nearly maximal.

10.30 a.m.   Convulsion lasting thirty to sixty seconds.   Rectum 42.2°C.   Blood sugar (right heart) 0.15 per cent.   The blood was obtained after respiration had stopped, and was dark.   The liver weighed 72.6 gm. and contained 0.05 per cent of glycogen.   The adrenal fragment weighed 0.060 gram.   The spleen was very large, 22.7 grams.   Necropsy otherwise negative.

Only in two of the partially adrenalectomised cats was a sufficient store of glycogen found to fulfil the condition necessary, according to the previous investigations, for a definite hyperglycemia.   In these five to six weeks had elapsed since the last operation.   In both experiments morphine yielded a negative result as regards the blood sugar.   In one cat (627) the hyperthermia and the general symptoms were well marked, but asphyxia, possibly because more moderate than usual, only caused a slight increase in the sugar content of the blood.   The liver contained 2.5 per cent of glycogen, which is generally quite enough to permit a good hyperglycemia with asphyxia.   But as pointed out in previous papers (6), it must be recognised that there is no hard and fast limit of glycogen content, and at present we do not know enough to allow for individual variations in the animals.   In the other experiment (cat 639) the animal was in very good condition. Its liver was of great size, and contained at the end of the experiment nearly 4.4 per cent. of glycogen.   Asphyxia (more severe than in cat 627) caused a marked hyperglycemia, whereas morphine seemed to be absolutely negative.   There is only one unsatisfactory feature in the experiment, the maximum rise of temperature was one of the smallest in the whole series (about 1°C.) and the general symptoms were not pronounced.   It is impossible to give any reason for this.   The dose of morphine per kgm. of bodyweight was considerably less than in cat 627, but still a dose which has usually elicited marked symptoms and a decided hyperthermia.

Cat 627; female; weight 1.6 kgm.

January 10. Excised $\frac{3}{5}$ of right adrenal and on January 28, the whole of the left gland. Used thereafter for exercise experiments (as cat 498), terminating April 19. Weight on May 6, 2.31 kgm. On May 27, weight 2.22 kgm. Denervated the remnant of right adrenal.

July 1. Weight 2.34 kgm. Fed on special diet for one week.

8.15 a.m. Rectum 38.6°C., room 24.5°C. Pulse 240, respiration 116. Animal excited, pupils wide. Blood sugar (8.25 a.m.) 0.10 per cent.

8.30 a.m. Morphine (30 mgm.). At 8.33 defecated. At 8.50 a.m. crying, apprehensive, getting restless, pupils maximal.

9.00 a.m. Quite active. Rectum 39.4°C. Pulse 290 to 300, respiration 78. Blood sugar (9.10 a.m.) 0.08 per cent.

9.40 a.m. Pilomotor effects. Pulse too rapid to count, respiration 60. Rectum 40.9°C.

10.05 a.m. Rectum 41.7°C., pulse 208, respiration 56. Active. Reflexes exaggerated. Blood sugar 0.10 per cent.

11.00 a.m. Less active. Rectum 41.45° C.

12.00 m. Rectum 41.1°C. Pulse 225, respiration 64. Blood sugar 0.09 per cent.

1.00 p.m. Lying quiet; apprehensive, reflexes still increased. Rectum 40.45°C.

2.00 p.m. Quiet. Rectum 39.8°C. Blood sugar 0.09 per cent. After moderate intermittent asphyxia for twenty-five minutes, blood sugar (at 2.35 p.m.) 0.12 per cent.

The anal sphincter was relaxed all through the experiment, unlike the condition in most of the animals, so that the thermometer was easily inserted.

The liver weighed 67.2 grams and contained 2.5 per cent of glycogen. Much abdominal, renal and omental fat.

Cat 639; male; weight 3.27 kgm.

June 3. Excised right adrenal and $\frac{3}{5}$ of left, and denervated the remnant. On June 30, weight 2.81 kgm. July 15. On "special" diet for 20 days; ate well; weight 3.58 kgm. Condition excellent.

8.30 a.m. Rectum 39.0°C.; room 23°C. Pulse 192, respiration 44. Blood sugar 0.09 per cent.

8.40 a.m. Morphine (30 mgm.). Defecated at 8.44 and 9.15 a.m. At 9.10 a.m. rectum 39.4°C., pulse 190, respiration 42, pupils somewhat wider, no other symptoms. At 9.25 a.m. lying quiet, but sometimes crying. At 9.40 a.m. rectum 39.7°C., pulse 158 (strong), respiration 50; apprehensive.

10.10 a.m. Rectum 39.8°C., pulse 156 (strong), respiration 64; more active. Erection of penis when active. Blood sugar 0.10 per cent.

11.00 a.m. A little more active. Rectum 40.0°C., pulse 180, respiration 60, pupils not $\frac{3}{4}$ maximal.

11.45 a.m. Rectum 39.5°C., pulse 140, respiration 58. Not as active. Blood sugar 0.10 per cent. At 12.30 p.m., no change in pulse, respiration or symptoms.

2.15 p.m. Quiet, but apprehensive. Rectum 39.85°C., pulse 180, respiration 36. Blood sugar 0.10 per cent.

2.30 p.m. Morphine (25 mgm.). At 3.00 p.m. no change in symptoms, pupils somewhat wider. At 3.15 p.m., rectum 39.7°C., pulse 180, respiration 34. At 4.00 p.m., rectum 39.75°C. No change in pulse or respiration.

4.30 p.m. Rectum 40.05°C., pulse 176 (strong), respiration 36. Blood sugar 0.10 per cent. After 20 minutes asphyxia (more severe than in cat 627) blood sugar (at 5.00 p.m.) 0.28 per cent. A marked inhibition of the heart was caused by each spell of asphyxia.

The liver weighed 151.2 grams and contained 4.36 per cent of glycogen. The adrenal remnant, which had probably hypertrophied somewhat, weighed 0.187 gram. Considerable abdominal and omental fat. Spleen 10 grams.

Two experiments were made on adrenalectomised rabbits. In both the rectal temperature eventually fell somewhat after morphine. There was no increase in the blood sugar content. But in one of the rabbits (630), which was in poor condition, the glycogen content of the liver was exceedingly small, and asphyxia, which regularly causes hyperglycemia in adrenalectomised rabbits with a good hepatic glycogen store, gave a negative result. In the other rabbit (638) the liver contained over 1 per cent of glycogen, and asphyxia caused a moderate hyperglycemia, whereas none was caused by morphine. It has been seen, however, that with this amount of glycogen some of the hyperglycemias previously studied (6) may fail to be elicited in certain animals, normal as well as adrenalectomised. So that the experiments on rabbits leave us at present in the same position as those on cats; and more work, which is now under way, is necessary before it can be stated whether morphine hyperglycemia is less easily obtained in the absence of the adrenals.

Rabbit 630; female; weight 1.88 kgm.

April 4. Excised right adrenal, and on May 5 excised the left. Weight 1.87 kgm. No special feeding with carrots or dextrose.

July 6. Weight 1.38 kgm., somewhat emaciated. 9.30 a.m. Rectum 39.45°C.; room 29° to 30°C. Pulse very fast, respiration 158. Blood sugar (9.40 a.m.) 0.08 per cent.

9.45 a.m. Morphine (50 mgm.). At 10.15 a.m. quiet and sleepy.

10.25 a.m. Rectum 39.65°C. Pulse 106, respiration 24. Blood sugar (11.45 a.m.) 0.10 per cent.

11.50 a.m. Rectum 39.45°C. Pulse 136 to 150 (irregular), respiration 44. Blood sugar (12.00 m.) 0.10 per cent.

1.00 p.m. Rectum 38.9°C. Pulse feeble. Respiration 39. Blood sugar (1.15 p.m.) 0.075 per cent. After six minutes intermittent asphyxia the animal died, and blood obtained from the right heart contained 0.07 per cent of sugar. The liver weighed 33.7 gm. and contained only a trace of glycogen (not more than 0.01 per cent). Necropsy, nothing special. No accessory adrenals found.

Rabbit 638; male; weight 2.47 kgm.

April 4. Excised right adrenal, and on May 5, the left. Weight 2.36 kgm.

July 7. Weight 1.98 kgm. Had received carrots daily and dextrose in the water for five days, but had not regained its original weight.

8.20 a.m. Rectum 39.3°C.; pulse too rapid too count, respiration 160. Blood sugar (8.30 a.m.) 0.11 per cent.

8.35 a.m. Morphine (50 mgm.). At 9.00 a.m. Lying very quiet, respiration slower. At 9.30 a.m. rectum 39.45°C., pulse 160, respiration 12 to 20 (shallow).

10.15 a,m. Pulse and respiration the same. Rectum 39.3°C. Blood sugar (10.30 a m.) 0.11 per cent. Rectum (at 11.30 a.m.) 38.4°C.

12.30 p.m. Rectum 38.1°C. Pulse rapid and feebler, respiration 14 to 18 (Cheyne-Stokes), pupils somewhat wider; animal very quiet.

1.15 p.m. Rectum 37.8°C. Pulse and respiration the same. Blood sugar 0.09 per cent. After twenty-five minutes intermittent asphyxia, blood obtained from right heart (2.00 p.m.) contained 0.19 per cent of sugar. The liver weighed 49 grams and contained 1.32 per cent of glycogen.

In comparing the operated with the normal cats, it should be be noted that in the former at the time the observations on morphine (or exercise) were made the hair was still quite short over

the part of the skin (possibly amounting to a third of the whole surface) which had been shaved for the operations. This would of course tend to increase the heat loss in these animals, and therefore to diminish the rise of body temperature caused by an increased heat production. It is not possible to know whether this factor caused any material effect or whether it was eliminated by the temperature-regulating mechanisms. In two of the morphine experiments the denuded parts of the skin were artificially covered (cats 612 and 615). The hair grew much more rapidly in the rabbits, yet they showed a fall rather than a rise of temperature, in contrast to the great and rapid increase of temperature in the cats, despite the increased loss of heat from the shaved skin. This emphasizes still more the fundamental difference in the reaction of the temperature-regulating mechanism to morphine in the two animals.

It has been observed by one of us (7) that when the hair is removed from the greater part of the body of small animals (rabbits and guinea-pigs) by close clipping, or better by shaving, and the the animal is kept in an empty box so that it cannot cover itself, it may die even with an air temperature as high as 15° to 18°C. (particularly in the open air), and that sometimes in twenty to thirty hours. The rectal temperature sinks fast, notwithstanding almost constant involuntary muscular contractions. If the radiation from the bare skin be measured it is found to be far in excess of that from the hair-covered surface. The animal evidently loses heat more rapidly than it can produce it. If now, before the cooling has gone too far, the animal be placed in a warm chamber, the rectal temperature rises again, and it recovers completely. When the increased heat loss is less perfectly compensated, as when the animal, without artificial heating, is allowed to cover itself with straw or to herd with other animals, serious consequences are averted, but the rectal temperature may sometimes be seen to remain distinctly subnormal for a time. Loss of weight is very common, even when abundant food is supplied. It is not known whether, or to what extent, this may be a factor in the temporary emaciation seen in a good many of our animals after adrenal operations, although they were kept in very well heated

quarters.   Deprivation of food, even for a day or two, adds to the difficulty of compensating for the increased heat loss.   As only a summary of the results, without any of the experimental data, has hitherto been published, two typical experiments on guinea pigs will be cited.

Guinea pig; weight 684 grams.

August 27.   3.35 p.m.   Rectum 38.0°.   4.35 p.m., clipped all the skin except the head.   Rectum 37.0°.   Air 14.8°.

August 28.   10.15 a.m. 36.7°.   Seems well.   Grass for food had been put in, also dry grass to cover the animal.   Now removed the dry grass.   Air 14.2°.   Rectum 37.4°, 36.4° and 37.0° at 12.45 p.m., 3.15 p.m. and 6.00 p.m.   respectively.

August 29.   Weight 598 grams; 36.7° and 36.1° at 10.30 a.m. and 4.25 p.m.   Shaved about one-fourth of the skin.   Shivered a great deal.   Dried and warmed, but shivering continued.   At 5.35 p.m. rectum 35.1°, and at 6.00 p.m. 37.7°.   The animal was now quite dry. It was put into an empty box.   Air 15° at the open window.

August 30.   10.30 a.m.. 36.8°.   Constantly shivering.   At 11.30 a.m. shaved about half the surface.   At 11.50 a.m. rectal temperature was only 27.0°.   Warmed and throughly dried the animal.   At 12.05 p.m. 32.0°.   Wrapped in cotton.   At 12.50 p.m. 34.1°, and at 2.50 p.m. 36.5°.   Now put into empty box.   At 3.30 p.m. 35.9°.   Air 14.8°. Continual shivering; skin red.   At 4.00 p.m. 35.9°.

Temperature of shaved skin of back 33.35° (4.10 p.m.): shoulder 35.2°; belly 35.8°; rectum 35.3° (4.20 p.m.), and 36.3° (4.50 p.m.). Air 14.8°.

August 31.   7.10 p.m., rectum 35.9°.   Air 12.9°.   At 7.15 food given.

September 2.   10.30 a.m.   Found lying on its side.   Rectum 24.0°.   Air 13.8°.   Warmed the animal and wrapped it in cotton. At 11.30 a.m., rectum 28.5°, and at 12.00 m. 30.1°.   It can now eat grass, but the movements of mastication are irregular and badly coordinated.   At 12.55 p.m. rectum 34.4°.   Put in thermostat at 32°.   At 3.35, rectum 38.7°.   Eats grass, drinks water with avidity, also warm dilute cane sugar solution.   Considerably emaciated. Weight 483 grams.   The hair seems to have grown very quickly on the shaved parts.   At 3.35 p.m. it was exposed to ordinary temperature. At 4.20 p.m. rectum 33.4°, falling rapidly while the observation was being taken to 32.6°.   Shivered a great deal.   Eats grass eagerly. At 4.37 p.m. rectum 33.1°.   Left all night in the thermostat at 32°.

September 3. 10.30 a.m. rectum 38.9°. Very weak. Breathing quick and pulse rapid and feeble. Wrapped in cotton and put into box at air temperature with food, which it began to eat.

September 4. Found dead at 10.30 a.m. Weight 414 grams. The necropsy showed no signs of pneumonia.

In the other experiment to be given, special precautions were taken to prevent loss of heat for a day after shaving. The animal survived, although its rectal temperature seemed to remain for some time distinctly below the temperature at the beginning of the experiment.

July 17. 4.20 p.m. Rectum 39.7°. At 4.40 p.m. (after the skin was closely clipped) 39.2°. At 5.05 p.m. after lathering and drying the skin 34.7°. At 5.35 p.m. 35.8°. Air 19.6°.

July 18. 10.30 a.m. Apparently quite well. Rectum 36.7°. Air 16.8°. The skin felt warm compared with that of a normal animal. At 4.30 p.m. the posterior two-thirds of the body was shaved, and the guinea pig put at once into thermostat at 32° with food.

July 19. 11.40 a.m. Quite lively. Rectum 40.2°. At 11.45 a.m. removed from thermostat and put into a box. Air 16.8°. At 1.30 p.m. rectum 37.3°, and at 3.05 p.m. 37.1°.

July 20. 4.00 p.m. Apparently well. Rectum 36.8°. Air 19.0°.

July 21. 10.45 a.m. Rectum 37.0°, and at 3.50 p.m. 36.9°. Air 16.5°.

Thereafter the temperature was only taken occasionally: 36.7° (July 22), 37.2° (July 23), 37.5° (August 2). Perfectly well.

In some experiments the skin was not shaved and only a half or somewhat more of it was closely clipped. There was some shivering, especially when the animal was in a separate box. There probably was a temporary, moderate diminution in the internal temperature, and sometimes a marked loss of weight even when plenty of food was provided. But no serious consequences were observed. A condensed protocol of an experiment on a female guinea pig follows. To save space "Rectum" is represented by R and "Vagina" by V.

September 2. 11.20 a.m. R, 37.5°.—September 6. 12.45 p.m. R, 37.7°; V, 38.3°. Weight, 749 grams. V, 39.2° at 4.30 p.m. Clipped posterior half of body. R, 37.4°, V, 38.1°. Air, 16.6°.

September 7.   6.20 p.m. R, 37.°1, V, 37.6°.   Air 15.5°.   Weight 709 grams.

September 8.   11.30 a.m. R, 35.8°, V, 36.4°.   Weight, 686 grams. Clipped the same half of skin more closely.   R, 36.6°, V, 37.3° at 3.45 p.m.   Shivers a little.

September 9.   11.35 a.m. R, 37.8°, V, 38.2°.   Weight, 694 grams. Put into separate box with food at 12.40 p.m. R, 37.5°, V, 38.6° at 2.35 p.m.   Shivers continually.   Put in open air at 2.35 p.m. R, 37.8°, V, 38.3° at 4.15 p.m.

September 11.   11.05 a.m. R, 36.9°, V, 37.4°.

September 12.   12.15 p.m. R, 38.1°, V, 38.4°.   Continual spasms of shivering during temperature measurements.   The hair has grown very rapidly.   Weight 653 grams.

September 13.   11.40 a.m. R, 37.7°, V, 38.0°.

September 14.   3.55 p.m. R, 37.7°, V, 38.4°.   Weight, 604 grams. The thermometer passes rather farther into the vagina than into the rectum.

September 19.   3.30 p.m. R, 37.9°, V, 38.0°.   Weight 571 grams. The hair is now well grown on the clipped part.   Average growth since last clipping (eleven days) nearly 5 mm.

An even greater loss of weight was seen in a rabbit whose skin had been clipped without shaving, while the rectal temperature fluctuated only within the normal range.

September 6.   11.50 a.m. R. 38.2°.   Weight, 1256 grams.   Clipped half of the surface.   At 4.00 p.m. R, 38.7°.   Air 16.6°, temperature on the clipped flank 36.5°, on unclipped flank 32.7°.   Plenty of food.

September 7.   6.30 p.m. R, 38.5°.   Weight, 1194 gm.

September 8.   11.15 a.m.   Weight, 1169 grams.   R, 37.4°, and at 3.55 p.m. 37.6°.

September 9.   11.30 a.m.   Weight 1202 gm., R, 37.9°, and at 4.35 p.m. 39.1°.   Air 16.0°.

September 11.   11.10 a.m. R, 39.1°.—September 12.   12.30 p.m. R, 39.0°.   Weight 1184 gm.

September 13.   11.50 a.m.   Weight, 1132 grams.   Clipped most of the rest of the skin, not very closely.   R, 38.8° at 12.55 p.m. and 38.0° at 2.35 p.m.   Air 16.4°.   Skin of right buttock 35.3°.   Shivers constantly.   R, 38.1° at 3.55 p.m.   Skin of right buttock 35.7°.   Covered thermometer with a fold of loose skin, 35.7°.

September 14. 3.45 p.m. R, 38.5°. Skin in fold over buttock 35.9°, in fold over flank 37.7°. Weight 1104 grams. Shivers continually.

September 15. 10.20 a.m. R, 37.7°, fold of flank, 36.6°.

September 16. 10.55 a.m. R, 38.5°; flank fold, 37.5°. Air 15.2°. Weight 1056 grams. At 6.10 p.m., after being in open air (at 15.9°) for seven hours, R, 38.1°, flank fold, 36.9°. ·

September 17. 12.55 p.m.. R, 38.1°, flank fold, 37.6°. Weight, 936 grams.

September 19. 3.30 p.m. R, 38.3°, flank fold, 37,6°, and at 4.40 p.m. 37.8° and 37.8° respectively. Weight 888 grams.

September 20. 11 a.m. R, 38.0°; groin fold 37.6°. Weight, 863 grams. Seems quite well.

September 22. 12.20 p.m. R, 37.6°, groin, 37.4°.· Weight, 933 grams. At 3.15 p.m. R, 38.9°.

September 23 and 24. R, 38.5° (at 12.10 p.m.), and 38.8° (at 4.30 p.m.) respectively.

September 25. 12.00 m. R, 38.9° and at 3.10 p.m. 38.7°. Weight 926 grams.

September 27. 11 a.m. R, 36.6°. Shivers. Air 16.8°. Left outside window till 5.30 p.m.; rectal temperature was then 37.3°.

September 30. Found dead. Weight 676 grams. The necropsy revealed no obvious cause of death.

It is not known how direct a cause of death increased heat loss was in the fatal cases, nor whether some of the deaths were merely accidental. Not enough control experiments were made to show whether emaciation could have been prevented by greater attention to feeding and with a more suitable, and perhaps a more abundant diet. The object of the experiments was simply to determine roughly whether a gross interference with the protective covering would in these small animals cause striking effects, particularly in connection with the alleged fatal result of suppression of skin "functions" by varnishing. It scarcely needs to be pointed out that the existence of considerable areas of recently shaved skin is a factor which must always be taken account of in observations on the basal metabolism of animals, particularly small animals, after operations.

Since we were comparing certain reactions caused by morphine on normal and adrenalectomised animals, we thought it worth while to test the extraordinary statement of J. T. Lewis (8), in a quite recent communication, that the minimum lethal dose of morphine for decapsulated white rats is 400 to 500 times smaller than for normal rats. All we can say is that we have found no basis whatever in our own results for the statement. Our rats were allowed to survive for a much longer period after removal of the adrenals, and were in no obvious respect different from normal white rats.

A female rat was decapsulated on April 18. It then weighed 216 gm. On July 6 it weighed 234 grams. It received 10 mgm. of morphine (0.043 mgm. per gram) subcutaneously, about one-tenth of the commonly accepted minimum lethal dose. A small dose was given purposely, as it seemed incredible from the assertion of Lewis that a considerable difference in resistance should not exist. In four to five hours the effect was beginning to wear off, and the animal was normal by the next morning. Twelve days thereafter the rat, now weighing 236 grams, received 75 mgm. (0.32 mgm. per gram). Twenty-four hours later it was fairly lively, but died at the end of twenty-eight hours.

A female rat was decapsulated on April 13. It weighed 208 grams. On July 7 it weighed 245 grams and received 50 mgm. of morphine (0.2 mgm. per gram). Next morning (twenty-four hours after the injection) the rat was active and apparently normal. On July 19, the body weight being 235 grams, the animal received 70 mgm. morphine (0.3 mgm per gram), and died in three hours.

A male rat, weighing 168 grams, was decapsulated by two operations (on April 12 and 25). On July 8 it weighed 225 grams. It received 100 mgm. of morphine (0.44 mgm. per gram), and died in two hours.

A control female rat, weighing 245 grams received 40 mgm. morphine (0.16 mgm per gram). Next morning (after twenty-four hours) it had fully recovered. Thirteen days thereafter, the weight being 233 grams, it received 70 mgm. morphine (0.3 mgm. per gram), and died within about four hours.

A control female rat, weighing 221 grams, received 100 mgm. morphine (0.47 mgm. per gram), and twenty-four hours later had apparently recovered. Twelve days thereafter, the body weight being 203

grams, it received 70 mgm. morphine (0.34 mgm. per gram). After thirty hours it had not entirely recovered but it was normal in forty hours.

A male control rat, weighing 178 grams received 90 mgm. of morphine (0.5 mgm. per gram), and died in about two and a half hours.

SUMMARY

The marked hyperthermia caused by morphine in cats (the rectal temperature increasing as much as 4°C.) has been studied in connection with the development of the general symptoms, the changes in the pulse and respiratory frequency, and the sugar content of the blood. It has been observed that there is no close association between the hyperthermia and the hyperglycemia, or even the degree of muscular activity. The hyperthermia and the general symptoms develop in the same way, and reach the same intensity in cats from which the greater portion of the adrenal tissue has been removed and the remaining fragment denervated, as in normal cats. Further experiments have been undertaken to show whether this is also true of the hyperglycemia.[1]

There is no foundation for the statement that adrenalectomised rats succumb to a very much smaller dose of morphine than normal rats.

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[1] It seems not to be true of the hyperglycemia, according to a series of observations reported by us at the New Haven meeting, December, 1921.

PERFUSION OF THE MEDULLA OF THE TERRAPIN (PSEUDOMYS TROOSTI) WITH ADRENALIN

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In a previous article (1) I reported results of experiments showing the action of adrenalin on the medulla of the turtle. The present experiments were prompted by the statement of Bush (2) that "epinephrin does not seem to exert a registrable influence on the cardio inhibitory center of the striped turtle."

The methods used in my previous experiments were changed slightly to meet the objections raised by Bush. After removing the plastron the cord was cut as low down as possible, well within the carapace, then all structures other than the two carotid arteries and vagi nerves were severed, the nerves alone being the only connectives between body and head since both carotids were tied off and a cannula inserted into one. Only those turtles in which inhibition of the heart could be produced by electrical stimulation of the vagi were used. Ventricular rhythm was recorded by attaching the apex to a recording lever. This method is essentially that used by Greene and Peeler (3), whom I should have credited in my previous article.

I used the yellow bellied terrapin (Pseudomys troosti) exclusively, having found that the map turtle (G. geographicus [Le Seura]) did not respond to vagus stimulation.

Ringer's solution was used as a diluent and for washing out the medulla before and after the drugs.

The solutions used were prepared from the tablet adrenalin of Parke, Davis and Company and made up immediately before perfusion. I found that in concentration of 1:10,000, oxidation is evident to the eye in about twenty minutes in an alkaline solution such as Ringer's.

131

Partial or complete inhibition was obtained in about 80 per cent of the experiments. ·With dilute solutions as 1:100,000, irregular slowing was produced. With 1:50,000 adrenalin com-
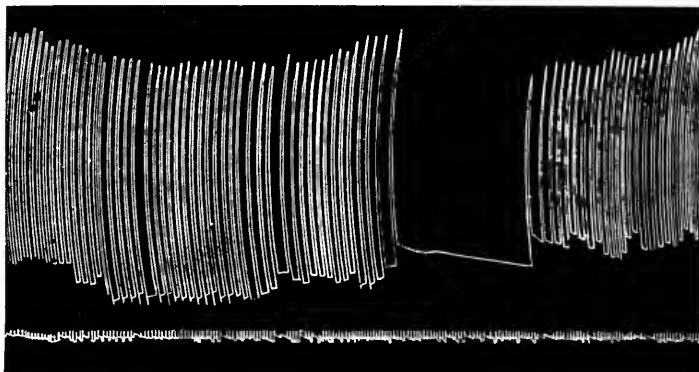
FIG. 1. PARTIAL INHIBITION PRODUCED BY PERFUSING THE BRAIN OF PSEUDOMYS TROÖSTI WITH 1 : 50,000 ADRENALIN

plete inhibition was produced in a number of cases as in figure 1. In this later experiment slowing became noticeable in three and one-half minutes and complete inhibition was evident
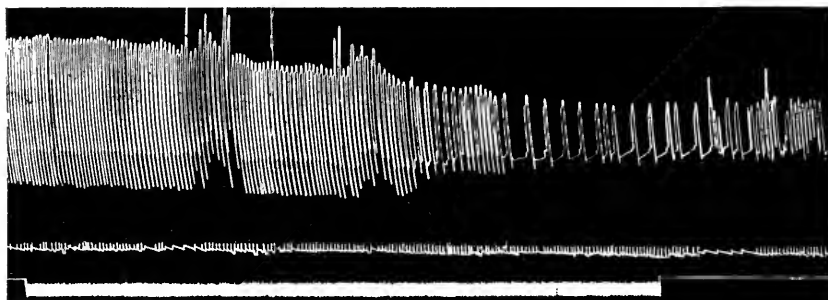
FIG. 2. COMPLETE INHIBITION EFFECTED BY THE PERFUSION OF 1 : 50,000 ADRENALIN THROUGH BRAIN OF PSEUDOMYS TROÖSTI

in five and one-third minutes respectively; total inhibition, however, did not follow. Total inhibition was not produced in the following experiments (fig. 2).

RATE	TIME	REMARKS
	minutes	
54		Normal with Ringer's
14	2	After 1 : 50,000 adrenalin
56		Before 1 : 50,000 adrenalin
22	$1\frac{1}{6}$	After 1 : 50,000 adrenalin

DISCUSSION

It is evident from the above experiments that adrenalin stimulates the cardio-inhibitory center of the Pseudomys troosti, producing slowing of the heart rate.

The statement of Bush that the spontaneous movements to minimize which I broke both femurs and humeri, were really strychnine convulsions effected by back seepage of the drug, is without basis.   This procedure was used to prevent irregular tracings.   In my first experiments the cord—the site of action of strychnine was destroyed.   Hence the movements were in all probability not due to the strychnine.

Bush's second objection that "since pithing the cord produces a not inconsiderable physiological disturbance, it is possible that a pharmacodynamic correlative must ensue" is hazy in meaning and is an assumption not warranted by the facts.   It makes little difference what is done with the cord after it has been cut, so long as the medulla which is the only part concerned in perfusion is left intact.   If spinal tone does influence the heart, then it is best that the cord be destroyed if only the influence of the cardio-inhibitory center is desired.   I have found that it makes absolutely no difference whether the cord below the section is destroyed or not.

The third assumption that unless all the tissues except the two carotids and vagi are severed possible influx of the drugs used into the general circulation may take place and there exert a "cardiocipetal" action is not justified in the case of adrenalin since this drug, as I and others have found, acts as a stimulant and increases both the rate and amplitude and hence the inhibition could not be attributed to any local action of the adrenalin. Complete section of all the tissues allows freer perfusion since the egress of the fluid is not hampered.

In these experiments I used the amphibian solution of Ringer, but I am inclined to believe that it does not make any difference whether Ringer's, Locke's, or normal salt is used. All my solutions were oxygenated sufficiently since the apparatus was so arranged that egress of fluid was accompanied by bubbling of air through the solution.

CONCLUSION

Adrenalin when perfused through the medulla of the yellow bellied terrapin (Pseudomys troosti) produces partial or complete inhibition of the heart-rate.

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THE INFLUENCE OF PURGATIVES UPON BLOOD CONCENTRATION

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The significant feature in the action of purgation is the watery character of the stools. The question naturally arises "what is the source of this fluid?" Two possibilities present themselves, viz., (1) the fluid is present because of lack of absorption of the intestinal contents induced perhaps by presence of the purgative, (2) the presence of the purgative draws water from the blood. It has been assumed for many years that the saline purgatives act according to the second hypothesis. On the other hand, the literature on this point is not very definite, especially with respect to detailed data. It is quite evident that if water is drawn from the blood to any considerable extent by the action of the purgative in the bowel, such an influence is capable of measurement by observation of changes in blood concentration.

In order to indicate the mode of action of different purgatives the present investigation has been so planned that blood concentration studies have been made upon the rabbit and dog receiving various types of saline and vegetable purgatives.

METHODS

For estimation of changes in blood concentration the content of hemoglobin has been determined, the method of Cohen and Smith being employed. Throughout this paper hemoglobin is expressed in percentage of the initial value rather than in terms of absolute value.

In order that conditions within the animal organism should

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[1] The data are taken from the thesis of Louis Errico presented in partial fulfillment of the requirements for the degree of M.D., Yale University, 1921.

135

be as constant as possible food and water were withheld for a period of twenty-four hours preceding the experiment. Blood was drawn from the ear vein. The purgative was administered through a stomach tube.

EXPERIMENTS WITH RABBITS

In the following table may be found data showing the variations in blood concentration encountered during a period of five hours for normal rabbits.

TABLE 1

Hemoglobin changes in normal rabbits

TIME	RABBIT A	RABBIT B
	Hemoglobin	Hemoglobin
	per cent	*per cent*
10.00	100	100
11.00	100	98
12.00	98	95
1.00	97	96
2.00	95	99
3.00	96	96

In six experiments varying doses of magnesium sulphate failed to elicit purgation. With the smaller doses the animals appeared unaffected. Larger quantities induced toxic symptoms indicating the absorption of the drug. Autopsy on one animal showed the stomach and upper intestine distended with fluid contents whereas the lower portion of the gut contained normal formed feces.

In general little or no influence upon blood concentration could be demonstrated. In Rabbit A of this series a definite marked concentration occurred without, however, the production of diarrhoea. The table illustrative of those experiments follows. It is probable that the failure of magnesium sulphate to produce purgation in the rabbit may be explained by the well-known fact that the stomach and intestine of this animal are so filled with material that the salt is too much diluted to exert much effect. It was therefore concluded that the rabbit is

an unsatisfactory animal for experiments of the character herein described.

TABLE 2

Hemoglobin changes in the rabbit following the administration of saline purgative

TIME	RABBIT A (20 cc. SATURATED $MgSO_4$)	RABBIT B (20 cc. $MgSO_4$)	RABBIT C (20 cc. SATURATED $MgSO_4$)
	Hemoglobin	Hemoglobin	Hemoglobin
	per cent	per cent	per cent
9.00	100	100	100
9.30	107	85	101
10.00	115	83	109
10.30	114	86	109
11.00	109	82*	99
11.30	100		95
12.00	90		

TIME	RABBIT A (20 cc. $MgSO_4$)	RABBIT C (30 cc. $MgSO_4$)	RABBIT D (40 cc. $MgSO_4$)
	Hemoglobin	Hemoglobin	Hemoglobin
	per cent	per cent	per cent
9.00	100	100	100
9.30	111	102	105
10.00	113	108	104
10.30	104	109	111
11.00	100	108	103
11.30	100	108	100
12.00		102	
4.00		100	

* Rabbit died.

EXPERIMENTS WITH DOGS

A. The influence of saline purgatives

The first passage of the stomach tube in a dog usually leads to vomiting. By repetition of the act the animal apparently overcomes this difficulty and even the introduction of a strong solution of a saline purgative does not produce vomiting.

The experiments herein recorded were carried through with magnesium sulphate, sodium sulphate and Rochelle salts. The results are illustrated by the graphs numbered figures 1 to 9.
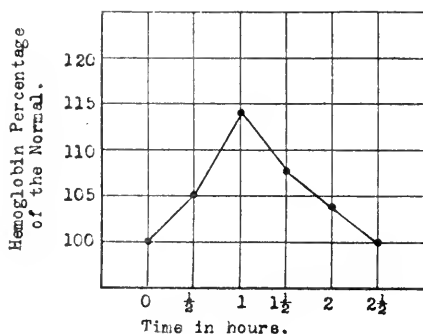
FIG. 1. DOG A

The influence of magnesium sulphate upon hemoglobin content. Animal received 40 cc. saturated solution of magnesium sulphate.
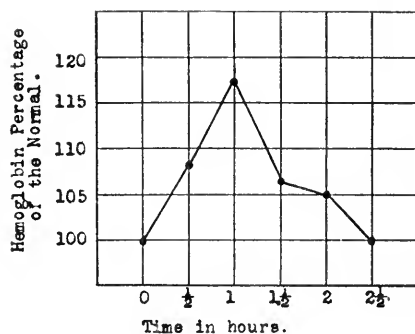
FIG. 2. DOG B

The influence of magnesium sulphate upon hemoglobin content. Animal received 40 cc. saturated solution of MgSO₄.
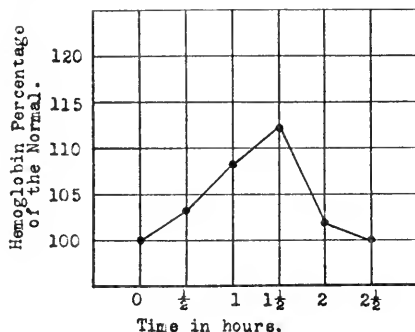
FIG. 3. DOG A

The influence of magnesium sulphate upon hemoglobin content. Animal received 40 cc. saturated solution MgSO₄.
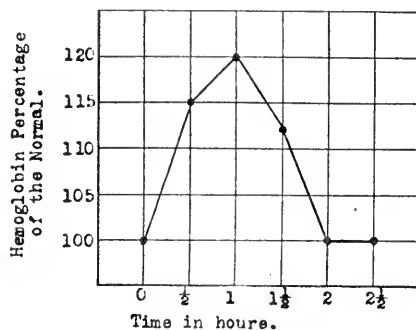
FIG. 4. DOG A

The influence of sodium sulphate upon hemoglobin content. Animal received 15 grams sodium sulphate in 100 cc. H₂O.
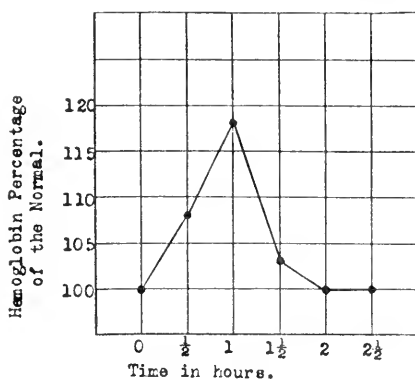
FIG. 5. DOG B

The influence of sodium sulphate upon hemoglobin content. Animal received 15 grams sodium sulphate in 100 cc. H₂O.
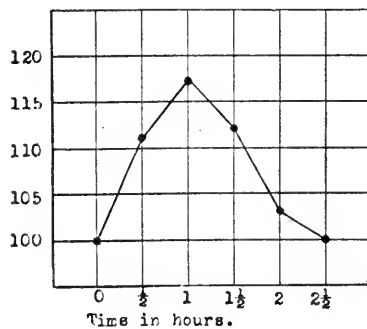
FIG. 6. DOG A

The influence of sodium sulphate upon hemoglobin content. Animal received 15 grams sodium sulphate in 100 cc. H₂O.

138

From inspection of the above graphs it is quite evident that magnesium sulphate (epsom salt) produced a distinct rise in the hemoglobin, which reached its maximum at about the second hour and returned to normal two and a half hours after adminis-
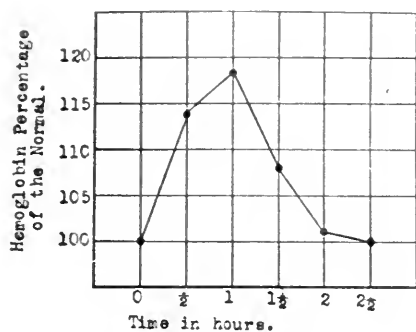
FIG. 7.   DOG B

The influence of rochelle salt upon hemoglobin content. Animal received 15 grams rochelle salt in 100 cc. H_2O.
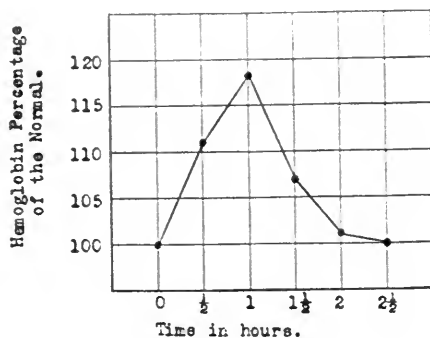
FIG. 8.   DOG A

The influence of rochelle salt upon hemoglobin content. Animal received 15 grams rochelle salt in 100 cc. H_2O.
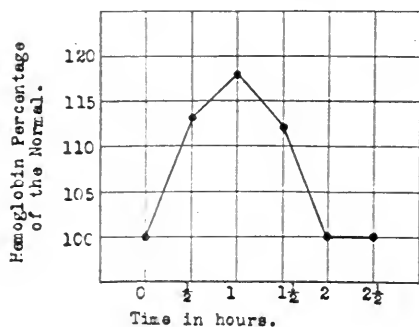
FIG. 9.   DOG B

The influence of rochelle salt upon hemoglobin content.   Animal received 15 grams rochelle salt in 100 cc. H_2O.

tration.   The same result was obtained with sodium sulphate and sodium and potassium tartrate (rochelle salt) except that the return to normal was about a half hour sooner.   Moreover the hemoglobin content has never gone above 120 per cent of the normal, the maximum having ranged between 114 per cent and
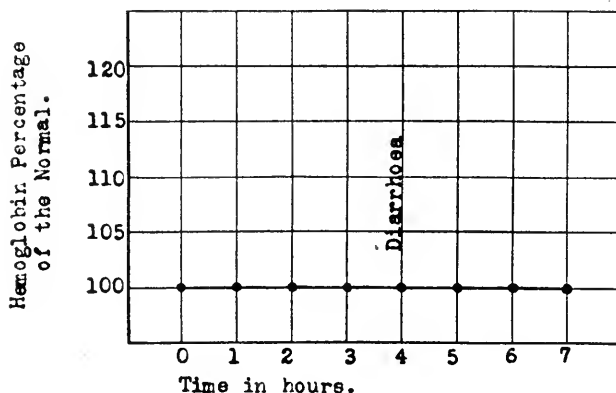
Fig. 10.   Dog A

The influence of castor oil upon hemoglobin content.   A nimal received 1 ounce of castor oil.

120 per cent of the normal.   This is worthy of note because Underhill in his book on "The Lethal War Gases" has shown that blood concentration is not of itself dangerous until it has reached a point above 125 per cent of the normal.

On the other hand, the fact that purgatives exert a definite influence in the direction of concentrating the blood indicates that
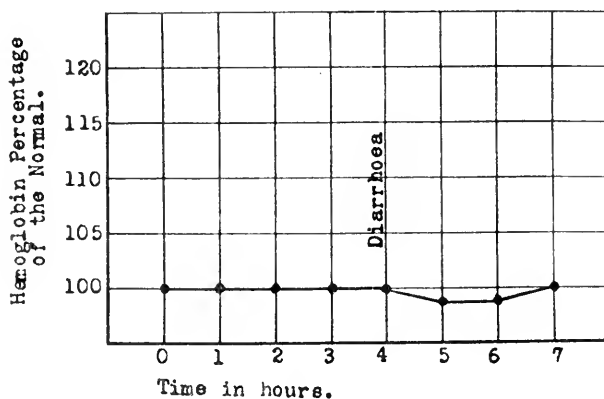
Fig. 11.   Dog B

The influence of castor oil upon hemoglobin content.   Animal received 1 ounce of castor oil.
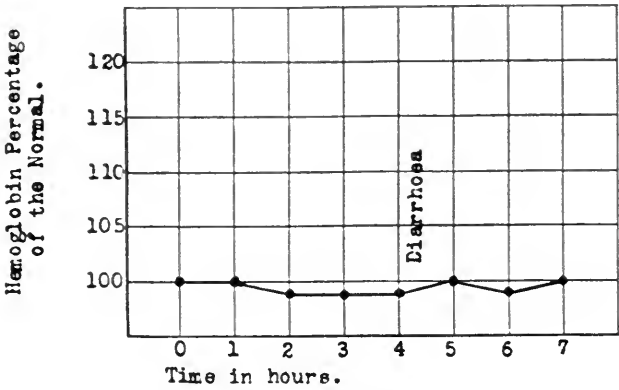
FIG. 12.   DOG A

The influence of castor oil upon hemoglobin content.   Animal received 1 ounce of castor oil.

some care should be exercised in the administration of purgatives in diseased conditions especially in those states known to be responsible for concentrated blood.   By ill-advised use of purgatives it is quite apparent that a blood concentrated to some extent and yet not sufficiently concentrated to be dangerous in itself may reach a dangerous concentration by the added influ-
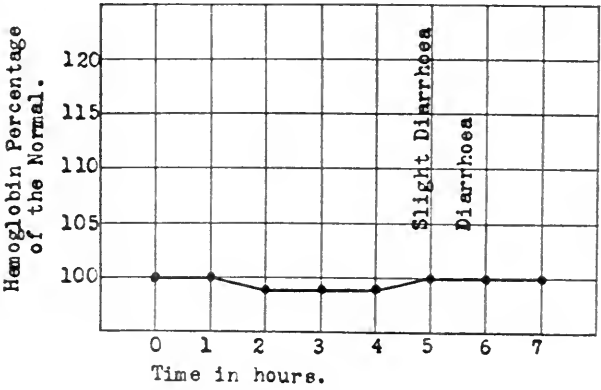
FIG. 13.   DOG B

The influence of cascara sagrada upon hemoglobin content.   Animal received 25 cc. of cascara sagrada.
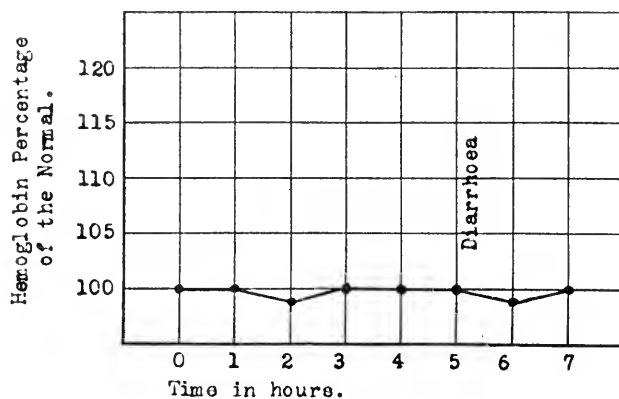
FIG. 14. DOG A

The influence of cascara sagrada upon hemoglobin content. Animal received 25 cc. of cascara sagrada.

ence of the purgative. Such a factor may account in part for the collapse which at times follows the administration of a purgative.

B. The influence of vegetable purgatives

The following experiments were carried through using castor oil and cascara sagrada and the results may be best illustrated by graphs numbered figs. 10 to 15.
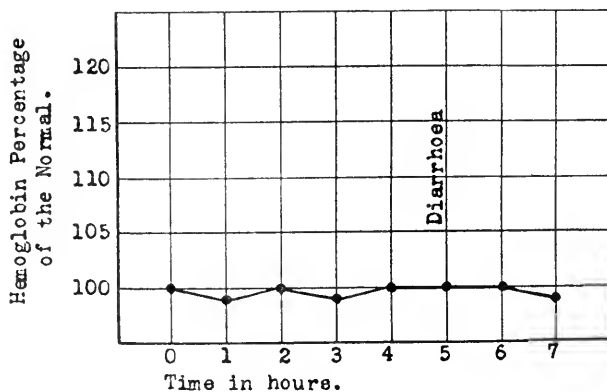
FIG. 15. DOG B

The influence of cascara sagrada upon hemoglobin content. Animal received 25 cc. of cascara sagrada.

The preceding graphs show clearly that castor oil and cascara sagrada produce no change whatever in the hemoglobin, although purgation occurred at about the fourth and sixth hour respectively.   These two groups of compounds, therefore, probably produce by local irritation a very active peristalsis which hurries the intestinal contents along the alimentary tract, preventing the absorption of the entire contents.

RÉSUMÉ

1. The hourly fluctuations in hemoglobin content of rabbits without purgation are relatively small and insignificant.

2. Rabbits are unsatisfactory animals for purgative experiments since they develop toxic symptoms with no diarrhea.

3. The saline purgatives produce, in dogs, a distinct concentration of the blood.   The maximum effect is reached during the second hour.

4. The vegetable purgatives produce no change in the hemoglobin content although diarrhoea occurs between the fourth and sixth hour.

5. The significance of blood concentration by purgatives is discussed in connection with pathological conditions.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF SOME PROTEIN DERIVATIVES

IV. THE TOXICITY OF VAUGHAN'S CRUDE SOLUBLE POISON[1]

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INTRODUCTION

Proteins when hydrolyzed either "in vivo" or "in vitro" are altered in their chemical composition whether it be due to the influence of acid, alkali or enzymes. The large protein molecules are reduced to smaller ones depending upon the degree of hydrolysis. Thus we have polypeptids of a high order, peptones, proteoses and eventually amino-acids. With the exception of the last mentioned all of these hydrolytic products are more or less toxic to animals when injected hypodermatically or intravenously. In protein intoxication it is quite important, therefore, to know the fate of these substances when introduced parenterally within the body tissues.

It is a well known fact that proteins upon first injection or upon frequent reinjection at short intervals of time, within ten days, seldom induce any severe physiological symptoms in animals. However, if the interval of time between injection and reinjection be extended to two weeks or more marked toxic effects are observed. Vaughan (1) explains these variations in action upon a basis of the rate of mobilization of the body

[1] The data of this paper have been taken from the thesis presented by Axel M. Hjort in partial fulfillment of the requirements for the degree of Doctor of Medicine, at Yale University, June 1921.

ferments and their speed of hydrolysis of protein into their toxic "Abbau Produkte." Under normal conditions the body tissues have a feeble fermentative action upon proteins. This is augmented by the introduction of proteins into the circulation. If the injections are frequent the ferments are not given an opportunity to mobilize sufficiently to produce a rapid hydrolysis of the proteins injected but if infrequent the ferment mechanism is given time to establish itself to a degree sufficient to handle the foreign substances in a short time, and it is this ability of rapid conversion into the toxic elements that is responsible for the severe physiological reactions thus manifested.

HISTORICAL

Vaughan (1) and his co-workers are in large part responsible for our present knowledge of toxic protein products. They observed that when proteins are hydrolyzed by either dilute acids or alkalies very toxic products result. They endeavored to ascertain the chemical constitution of these products but had little success. As a result no more is known at the present day about the chemical nature of these toxins than was known twenty years ago. Most of Vaughan's investigations were made with the toxins obtained by alkali hydrolysis of proteins, and to these are given the name: "Vaughan's Crude Soluble Poison."

Vaughan's crude soluble poison is prepared by digesting proteins with 2 per cent sodium hydroxide. By this mode of hydrolysis he found that it was possible to isolate two types of substances, (1) a non-toxic or carbohydrate and (2) a toxic or protein-like. These are both soluble in water and dilute alcohol, and are, therefore, difficult of separation. However, the latter is soluble in absolute alcohol, and the former not. To utilize this property of the poison to the best advantage Vaughan later prepared his poison by hydrolysis of the protein in absolute alcohol containing 2 per cent sodium hydroxide.

Vaughan's method. To a weighed amount of protein in a flask 15 to 25 times its weight of absolute alcohol containing 2 per cent sodium hydroxide is added. A reflux condenser is attached and the flask with its contents is heated on a hot water

bath for one hour.   At the end of this time the undissolved part of the protein is freed from the solution by filtration, and is again placed in the flask to repeat the process with a fresh portion of the alkaline alcohol.   In all, the protein is submitted three times to this treatment after which the filtrates are combined and neutralized with hydrochloric acid, avoiding an excess of the acid.   The precipitate resulting from the neutralization contains both sodium chloride and some of the base.   After neutralization the precipitate is removed by filtration, and the filtrate is evaporated in vacuo at 40°C. to dryness.   This is redissolved in absolute alcohol and filtered to remove traces of sodium chloride, after which the filtrate is again evaporated to dryness in vacuo at 40°C.   According to Vaughan the poison loses some of its potency, when evaporated under ordinary atmospheric pressure.

Pryer (2) later modified Vaughan's method somewhat in controlling the temperature of the hot water bath between 86° and 92°C. and in adding enough hydrochloric acid to pass the neutral point, to phenolphthalein, to the extent of making the extract one per cent or more acid.   A further modification adopted was to just drive off the alcohol and then dissolve the syrupy residue in water.   This aqueous solution was kept for injection purposes.   By his modification a product was obtained which was very much more potent than that made by Vaughan's method.   Comparing the toxicity of this preparation with that of Vaughan by intravenous injection into guinea-pigs he found that relatively enormous doses of the latter are necessary to kill.   His preparations varied in acidity from 3 to 6 per cent and enough of the poison was obtained from 10 grams of casein to kill 8000 guinea-pigs.

Vaughan succeeded in getting the poison from the following substances; egg-white, casein, serum albumin, edestin, zein Witte's peptone, Macquaire's peptone, de Chapoteaut's peptone, the tissues of cancer, and the cellular substance of B. coli communis, B. typhosus, B. anthracis, B. tuberculosis, B. moelleri (timothy), Sarcina lutea, B. ruber of Kiel, B. proteus, B. subtilis, B. megaterium, B. pyoncyaneus, B. pneumoniae and B. diphtheriae.   It was not obtainable from gelatin nor Defresnes peptone (3).

Vaughan describes his poison as a brownish powder varying in shade of color with the protein from which it is obtained. It has a peculiar odor. The dried product is highly hydroscopic and freely soluble in water, a property which also varies with the protein from which it is obtained, and possibly with the length of digestion with the alkaline alcohol. Whatever portion of the poison is insoluble in water is also devoid of toxic effect. Aqueous solutions of the poison are decidedly acid to litmus. Part at least, of this acidity he describes as of organic origin for mineral acids precipitate aqueous solutions, a property characteristic of organic acids. Neutralization with sodium bicarbonate precipitates a non-toxic element. Incubation with an excess of alkali diminishes its potency, which he thinks is caused by the formation of a salt with the acid poison and the alkali. The poison is soluble in methyl as well as ethyl alcohol, but a non-toxic portion of the alcoholic extract is insoluble in absolute alcohol. It is insoluble in ether, chloroform and petroleum ether.

The poison gives all the important protein color reactions with the important exception of that of Molisch. The positive tests are the Millon, biuret, Adamkiewicz and the Liebermann. It does not reduce Fehling's solution even after prolonged boiling with dilute mineral acid. Sulphur is present. Heat does not coagulate the poison whether it be in acid, neutral or alkaline solution. It diffuses slowly through collodion sacs. A precipitate forms with the heavy metals and also with phosphotungstic, phosphomolybdic and picric acids. Altho these reactions are characteristic also of alkaloids none of them could be isolated. Aqueous solutions of the poison are only in part salted out by ammonium sulphate and sodium chloride.

Pryer (2) later endeavored to determine the cause of the acidity of the poison. He concludes after shaking an aqueous solution with silver carbonate, with the resulting slight diminution of its acidity, that the acid character of the poison is only in part due to hydrochloric acid.

The physiological effects manifested by guinea-pigs after injection with the poison are described by Vaughan as follows: The first stage which occurs in five to ten minutes after injec-

tion is indicative of excitement and peripheral nerve irritation. Five minutes later the temperature begins to fall and sometimes reaches 94°F. or even lower within a half hour. The animals then begins to show signs of incoördination, which is rapidly followed by partial paralysis, especially marked in the hind extremities. This stage lasts for from five to ten minutes, during the latter part of which the animal lies quietly on one side. From this condition the animal passes into what may be called the convulsive stage, during which there are observed clonic convulsions, at first involving only the neck muscles. In the beginning of this stage the convulsions are slight and very infrequent, but later they gain in intensity and frequency, and eventually become general, involving the body muscles. This stage when present presages a fatal outcome, and it is rare for an animal to recover from it. During a convulsion, or occasionally in the interval of calm, the respiration ceases, altho the heart continues to beat 3 to 4 minutes longer. The fatal issue, if it occurs at all, always results within an hour after injection, and usually within from thirty to forty minutes. This is to a large extent independent of whether the dose is the minimal lethal or three to four times that amount. It is certainly entirely independent of the size of the pig. Death occurs at slightly different times with different batches of the poison, but even in this case the interval of time between the injection and the fatal issue does not vary to any great extent. A dose which has proved to be minimal fatal for one pig will almost surely prove to be the same for another.

Neutral and faintly alkaline solutions of the poison invariably kill pigs in doses of 60 mgm., but strongly alkaline preparations of the same amount do not cause a fatal result, altho the animals are very ill. The alkali evidently alters the poison in some way. Intraperitoneally 8 to 60 mgm., of the poison is lethal to guinea-pigs. A preparation which proves lethal in doses of 60 mgm., intraperitoneally, will require 120 to 180 mgm. for the same result subcutaneously, and but 10 to 15 mgm. intravenously. In the last mentioned case death ensues much sooner than in the others. As previously mentioned Pryer (2) found the strongly acid preparations much more potent than the others.

The physiological symptoms induced by injections of Vaughan's crude soluble poison closely simulate those of histamine. Pryer (2) compared them by guinea-pig injections but concluded that the toxic element of the former was not histamine. Choline and its derivatives closely simulate the poison in their actions. Pryer (2), however, was unable to isolate any choline derivative from the poison. With the hope of finding some amino-acid or group of amino-acids which could be held responsible for the action of the poison the same author injected leucin, glycocoll, alanine, histidine, tyrosine, phenylalanine, glutaminic acid, aspartic acid and tryptophane intraperitoneally into guinea-pigs with negative results. He then administered a mixture of alanine, phenylalanine, leucine, glycocoll, tryptophane, glutaminic acid, histidine, tyrosine, uric acid and glucose with like success. He concludes that the poison is not a simple chemical compound, but is a protein change product, acid in reaction, capable of forming salts, and reacting much like globulins in its behavior toward neutral salts.

The foregoing authors have, therefore, succeeded in preparing very toxic products by the hydrolysis of a number of proteins, which are similar in action. The exact nature of the poison has as yet not been determined, altho it is similar in action to some definite chemical substances. It is this last mentioned fact which has particularly interested the present authors.

EXPERIMENTAL

I. Preparation of Vaughan's crude soluble poison

All preparations although carefully prepared by the same method do not have the same toxicity according to Vaughan and Pryer. Accordingly several preparations were made by their methods, and others by variations in the method which will be mentioned in their proper place.

Preparations A and B were made exactly according to Vaughan's directions with the one exception that evaporation was done at ordinary atmospheric pressure. In each case 50 grams of casein was submitted to the alkaline alcohol hydrolysis

as described by Vaughan.   Twenty-five times its weight of absolute alcohol containing 2 per cent sodium hydroxide was used for each extraction.   After extraction the combined filtrates of each preparation were neutralized to litmus with concentrated hydrochloric acid.   After filtration the neutral extracts were evaporated to dryness on hot water baths, redissolved in absolute alcohol, again filtered to remove traces of sodium chloride and then evaporated to dryness.   On dissolving the dried preparations with water and titrating weighed amounts for the degree of acidity, using phenolphthalein as indicator, preparation A was found to be alkaline, equivalent to 0.43 per cent sodium hydroxide, and preparation B acid, equivalent to 0.67 per cent hydrochloric acid.

Preparation C was made by the method described by Pryer, except that the neutral point was not passed quite to the extent of 1 per cent acid.   One hundred grams casein was digested by three 1500 gram portions of absolute alcohol containing 2 per cent sodium hydroxide.   The combined extracts were made faintly acid, and then were filtered.   The filtrate was evaporated on a hot water bath at ordinary atmospheric pressure until the alcohol was driven off.   The residue was redissolved in absolute alcohol to remove traces of sodium chloride.   After filtration and reevaporation, the residue was dissolved in water, the insoluble portion being filtered off, and the filtrate set aside for later use. Its acidity was 0.7 per cent which was increased to 1.09 per cent by the addition of hydrochloric acid.   The acidity of this preparation in terms of its solid content was approximately 10 per cent.

Preparation D was prepared by Vaughan's method including evaporation in vacuo at 40°C. but the neutral point of the combined extracts was passed to the extent of a faint acidity.   The dried product in this case revealed an acidity of 9 per cent.

II. *The toxicity of Vaughan's crude soluble poison*

When these preparations were injected intraperitoneally a variation in toxicity, as determined by the minimal lethal dose, was observed.   This substantiates Vaughan's findings.

In our records we have divided the fatalities into two groups, (1) the primary, occurring within two hours, and a respiratory death, (2) the secondary, death within thirty-six hours and following a progressive esthenia, which is cardiac in nature. See tables 1 and 2 of Appendix.

Preparation D, tho not entirely soluble proved the most toxic. A primary lethal outcome resulted on the two animals receiving 150 mgm. of this preparation per 350 grams of body weight. Preparation C followed in degree of toxicity for three out of six pigs receiving 150 mgm. as above did not survive, and in these cases the death was primary. In the case of preparation A the same dose proved fatal in two out of three pigs, but none of the deaths was primary. In preparation B only one pig out of three succumbed, under the previously mentioned conditions, and that one died a secondary death.

When comparing the toxicity of the above preparations it is well to refer to their chemical reactions and mode of making. Preparations C and D have about an equivalent acidity, and the difference in their toxicity may be explained upon the basis of the observation made by Vaughan that evaporation at ordinary atmospheric pressure somewhat reduced their toxicity. D was made by evaporation in vacuo at 40°C. but C was not. In the case of preparation A and B it is difficult to explain their variation, for the one which is slightly alkaline proved more toxic than the other. It is possible that a greater series of injections might favor the acid preparation, but their chemical reactions approximate so closely the neutral that it is doubtful whether a trace of alkali or acid would modify their toxicity to any great extent.

III. *The preparation of Vaughan's crude soluble poison of higher acidity*

The strongly acid preparations proved to be the most toxic. This naturally leads to the question as to whether the acid itself plays a rôle in the toxicity of the poison. Pryer's observations regarding the greater toxicity of his acid preparations over the neutral also point to the importance of acid in the highly potent poisons. However, there is one other possibility in the case of

Pryer's acid preparations which may play a part in the potentiation of its toxic effect, and that is the factor which heat plays in evaporating down a progressively concentrating acid solution of the poison.

With all of these questions in mind several more preparations were made. Two grams each of preparation A, B and D were dissolved in 150 cc. portions of 1 per cent hydrochloric acid in absolute alcohol and the alcohol was in each case evaporated and the syrupy residue redissolved in water. The insoluble portions were removed by filtration and the filtrates set aside for further use as preparations A', B', and D.' To obtain a preparation of higher acidity and simultaneously observe the effect of evaporation of a highly acid poison on its toxicity B was dissolved in 500 cc. of 1 per cent hydrochloric acid in absolute alcohol, the alcohol evaporated off, and the residue again dissolved in water, with subsequent separation of the insoluble portion. But one further preparation was made and in that case the neutralization precipitate obtained in preparations A and B was extracted with 500 cc. 1 per cent hydrochloric acid in absolute alcohol, after which the alcohol was removed by evaporation and the residue dissolved in water. See tables 1 and 2 of Appendix.

IV. The influence of the acid on the toxicity of Vaughan's crude soluble poison

The acidity of preparations A', B', D', A'B' and B'' was 0.94, 0.82, 0.96, 2.48, 5.47 per cent respectively. When these preparations were injected intraperitoneally in guinea-pigs the following results were obtained. Four pigs succumbed to 150 mgm. per 350 grams weight of preparation A', one by primary and the others by secondary effects. In B' two out of four succumbed, one being by primary lethal effect. Preparation D' failed to kill any of the four pigs injected with the same dose as in the previous poisons. B'' killed five of the six pigs injected in doses of from 27 to 120 mgm. but none by primary action. A'B' proved fatal to one pig in a dose of 100 mgm. by secondary effect.

Although preparations A and B which were practically neutral were the lowest in toxicity, they gained considerably in potency by being made acid, A′ and B′. In the case of D′ it is difficult to explain the diminution in toxicity as compared with D, for the acidity, the former 0.96 per cent in solution and the latter 9 per cent as a solid, is approximately equal as administered. In the preparation of D′ some material separated out insoluble in water, and the alcoholic solution was evaporated at ordinary atmospheric pressure. Both of these factors may have played a part in the decrease in toxicity. Evaporation in concentrating acid evidently does not increase the toxic action, when given intraperitoneally, for B″ was no more potent than any of the others, for occasionally other preparations prove lethal at 100 mgm. and, furthermore, the deaths in this case can be accountable to the acid content itself as will be seen later. Likewise making the combined extracts acid to the extent of 1 per cent does not remove from them more potent material than does the addition of acid just to the point of neutrality, for A′B′ is no more toxic than A and B.

V. The source of the acidity of the poison

The acid content of the poison does seem to add some to its toxicity, but is not entirely responsible for it. As was previously mentioned Vaughan and Pryer think that hydrochloric acid is only in part the cause of the acid reaction of the poison. From our experience the acidity appears to be entirely dependent upon the hydrochloric acid added for the purpose of neutralization for when the combined alkaline alcohol extracts are carefully neutralized to litmus the resulting dry poison is either faintly alkaline or acid as demonstrated by preparations A and B. However, if the extracts are made faintly acid, the ultimate product is more acid, and this acidity increases with the degree to which the neutrality is passed on the acid side. Preparations C, D, B″, A′B′, A′ and B′ demonstrate this fact. The acidity is mostly free, for in titrating the acid poison first against Töpfer's solution, and then phenolphthalein, 13/14 is free.

VI. *The toxicity of hydrochloric acid*

Since hydrochloric acid seems to be the acid element of the poison, it is important to study the effect of the acid on guinea-pigs when injected intraperitoneally in varying concentrations, Table 3 Appendix. The lethal effect of this acid varies with its quantity and concentration. Ten milligrams kills in ten hours, 20 to 50 milligrams in 1 per cent solution are fatal in ten hours, 100 milligrams in 2 to 3 per cent solution proves lethal in one and one half to two hours. Greater amounts kill in about the same time as the last mentioned. It is evident from these results that the fatalities in preparations B″ and A′B′ can be entirely accounted for by the acid content.

Pryer found the higher toxicity of his acid preparations by intravenous injections. To further substantiate the view that the high acidity alone may be responsible for these results two pigs were given 0.5 cc. B″ each intravenously and two others 0.5 cc. 4 per cent hydrochloric acid by the same route. One of the first series almost succumbed in a few minutes but ultimately recovered. The other suffered little ill effect. The acid did nothing more than whip up the respiratory rate to some extent. In these experiments a highly acid preparation proved not to be very toxic, for it did not kill in doses of Vaughan's upper limit, 50 milligrams, likewise the acid itself did not prove lethal. It is difficult, therefore, to account for the variation in toxicity between Pryer's acid and neutral preparations.

VII. *The influence of neutralization upon the toxicity of the poison*

As a final step for comparison between the acid and neutral preparations with regard to toxicity a surely fatal dose of preparations C and D′ was determined, namely 300 mgm. Of three injections of each two fatalities were primary and one secondary. After neutralizing there were two primary fatalities and two recoveries in the case of C, and three recoveries out of three injections in D′. D′, however, precipitated slightly on neutralization which may offer a criticism of the results derived from it. In the case of C a surely fatal dose is converted into

an uncertain one, which indicates further the importance of the acid to the toxicity of the poison.   Furthermore we have been unable to prepare any poison with as high a toxicity as those described by Vaughan.

VIII. The toxicity of other products of protein hydrolysis and their derivatives

Earlier in this paper the question of the products of protein hydrolysis was discussed.   Amongst these were included peptones and proteoses.   Vaughan's crude soluble poison, therefore, is made by a process similar to the one responsible for the formation of these substances.   It is possible that it may be of the nature of one of them.   This matter let to an examination of the systemic effects of Witte's peptone and pure deutero-caseose, as well as some of their extracts.

a. Witte's peptone and its derivatives.   Table 4 of Appendix. Witte's peptone is not very toxic, as doses ranging from 200 to 600 mgm. failed to induce any physiological effect other than a little depression.   An alcoholic extract of this peptone did not kill in a dose of 300 mgm.   When, however, an extract of Witte's peptone was made as follows, the toxicity was high: To 25 grams Witte's peptone 500 cc. of hot 1 per cent hydrochloric acid in absolute alcohol was added and the mixture was shaken well for five minutes, after which it was allowed to stand to cool for one hour.   It was then filtered and evaporated to dryness.   It was very hydroscopic and grayish brown in color, and about a 40 per cent yield was obtained.   When 150 mgm. was injected in a pig very severe symptoms, typical of Vaughan's crude soluble poison were induced, but the pig ultimately recovered.   One pig of four given 200 mgm. died in thirty minutes. In doses of 300 mgm. death was certain and very acute.   The toxicity of this poison was very much the same as that of Vaughan's.   That this toxicity was due not alone to the acidity but to some alteration of the peptone was proved by neutralizing the acid extract before evaporation.   After neutralization a process which removed the greater part of the base by precipitation with the salt, the precipitate was removed by filtration,

and the filtrate evaporated to dryness.   Four hundred mgm. of this material failed to induce any symptom other than depression. Neutralization of the dry acid preparation which was very poisonous before injection precipitated some material which invalidated the results therefrom obtained.   Acid alcohol, therefore, extracts from Witte's peptone a substance which when evaporated to dryness in acid solution is very toxic, in fact comparable to the poison described by Vaughan.

b. Pure deuterocaseose and its derivatives.   Table 4 of Appendix

Pure deuterocaseose reacts similar to Witte's peptone in toxicity, both in the ordinary form and in the acid extract.   This proteose does not kill nor induce typical toxic symptoms in doses up to 500 mgm.   The acid preparation, made by extracting 5 grams of the pure caseose with 500 cc. hot 1 per cent hydrochloric acid in absolute alcohol for 5 minutes and then setting aside to cool for an hour, after which it was filtered and the filtrate evaporated to dryness, was hygroscopic and chocolate brown in color. The yield was from 40 to 50 per cent.   Doses of 300 mgm. were surely fatal intraperitoneally in guinea-pigs.   However, when, as was done in the case of Witte's peptone, the extract was neutralized before evaporation, the final dry product did not induce even the typical symptoms in doses of 400 mgm.   The acid itself of the acid preparation was not the cause of the high potency, for when this neutral extract was injected with 40 mgm. of hydrochloric acid none of the typical symptoms were observed and the animal died a secondary death in a time which could be accounted for by the acid alone.   Acid alcohol extracts from the pure deuterocaseose a substance which is very toxic when evaporated to dryness in acid solution.

IX. The influence of dilute Alkali on the nitrogen content of Vaughan's crude soluble poison

Vaughan, as mentioned previously, noticed a diminution in toxicity of his poison when it was incubated with sodium bicarbonate.   He accounts for this by the assumption that a salt is formed between the acid poison and the alkali.   The nitrogen

contents of Vaughan's poisons are very much the same in the various preparations made by us, table 1 of the Appendix. If the nitrogen contents resemble each other closely it is possible that loss of nitrogen might be instrumental in decreasing the toxicity of the poison. This Vaughan noted when he heated the alkali alcohol extracts for too long a time with loss of ammonia. To determine whether or not incubation of the poison at 38°C. for a week with 0.5 per cent sodium hydroxide results in loss of nitrogen, a weighed amount was placed in a volumetric flask and autoclaved, enough (calculated) sterile sodium hydroxide was added to make 0.5 per cent up to volume. Check analyses were made by the Kjeldahl method before and after incubation. No sign of bacterial growth was observed. The nitrogen contents before and after the weeks incubation were 10.92 and 10.88 per cent respectively. Evidently an inappreciable quantity of nitrogen was lost, for the results lie within experimental error.

Loss of nitrogen cannot, therefore, be the cause of the diminution in toxicity of the poison when it is incubated in weakly alkaline solutions.

CONCLUSIONS

1. Alkaline hydrolysis of casein in absolute alcohol produces a substance having a toxic effect which proves lethal when injected intraperitoneally into guinea-pigs in doses ranging from 100 to 300 mgm.

2. There is a considerable variation in the toxicity of the different preparations of Vaughan's crude soluble poison.

3. The fatal outcome following the injection of the poison occurs in two ways, (1) a primary, due to respiratory failure within two hours following its administration, and (2) a secondary occurring in 2 to 36 hours as the result of a progressive asthenia.

4. The preparations having the greater acidity prove to be the most toxic.

5. The acid content of the poison is not alone responsible for its toxic action.

6. The greater part of the acidity of Vaughan's crude soluble poison is uncombined hydrochloric acid.

7. The degree of acidity of the poison depends upon the excess of acid added in the process of neutralization of the alkaline alcohol extract, and the extent of heating while evaporating to dryness.

8. The decrease in toxicity of the poison subsequent to incubation in faintly alkaline solution is not due to a loss in its nitrogen content.

9. Hydrochloric acid when injected intraperitoneally into guinea-pigs proves fatal in doses of 5 mgm. or more, the acuteness of the death depending upon its quantity and concentration.

10. Acid alcohol extracts from Witte's peptone and pure deuterocaseose substances which when evaporated to dryness in acid alcohol solution are very toxic, and closely resemble Vaughan's crude soluble poison both in action and degree of potency.

11. Vaughan's crude soluble poison differs from Witte's peptone and proteose in that its toxicity is not so greatly modified by evaporation in acid alcohol solution as obtains for the latter two.

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APPENDIX

Throughout the animal experimentation aseptic technique was followed. The guinea-pigs varied in weight from 300 to 450 grams.

TABLE 1

The toxicity of Vaughan's crude soluble poison when injected intraperitoneally in guinea-pigs

PREPARATION	HYDROCHLORIC ACID CONTENT	NITROGEN CONTENT	SOLID CONTENT	27-100 mgm.		100 mgm.		125 mgm.		150 mgm.		200 mgm.		300 mgm.	
				F	R	F	R	F	R	F	R	F	R	F	R
	per cent	*per cent*	*per cent*												
V. C. S. P.—A..	0.43 (NaOH)	11.20	100			0	2			2	1	1	0		
V. C. S. P.—A'.	0.94	12.77	6.40					0	1	4	0				
V. C. S. P.—B..	0.67	11.30	100			1	1			1	2	1	0		
V. C. S. P.—B'.	0.82	12.23	5.79					1	0	2	2				
V. C. S. P.—C..	1.09	12.03	8.26			1	2			3	3	1	1	3	0
V. C. S. P.—C..	Neut.													2	2
V. C. S. P.—D..	9.20	11.80	100			0	2			2	0				
V. C. S. P.—D'.	0.96	12.31	7.72					0	1	0	4	0	2	3	0
V. C. S. P.—D'.	Neut.													0	3
V. C. S. P.—A' B'............	2.48	11.80	5.46			1	0								
V. C. S. P.—B''	5.47	13.60	5.49	3	0	1	1	1	0						

V. C. S. P. A = Alkaline 0.43 per cent in terms of NaOH.

V. C. S. P. C and D' (Neut.) = Acid preparations neutralized with NaOH. Some precipitate formed in the latter.

F = Fatal cases within thirty-six hours after intraperitoneal injections.

R = Recoveries at end of thirty-six hours following the injections.

TABLE 2

Supplementary to table 1; only fatalities are herein recorded

PREPARATION	DOSAGE PER 350 GRAMS											
	27–100 mgm.		100 mgm.		125 mgm.		150 mgm.		200 mgm.		300 mgm.	
	P	S	P	S	P	S	P	S	P	S	P	S
V. C. S. P.—A			0	1			0	2	0	1		
V. C. S. P.—A′							1	3				
V. C. S. P.—B			0	1			1	0	0	1		
V. C. S. P.—B′					1	0	1	1				
V. C. S. P.—C			1	0			3	0	1	0	2	1
V. C. S. P.—C (neutral)											2	0
V. C. S. P.—D							2	0				
V. C. S. P.—D′											2	1
V. C. S. P.—D′ (neutral)											0	0
V. C. S. P.—A′B′			0	1								
V. C. S. P.—B″	0	3	0	1	0	1						

P = Primary fatalities, occurring within two hours following the injection. Death is respiratory.

S = Secondary fatalities, occurring in from two to thirty-six hours after the injection. Death due to progressive asthenia and probably circulatory failure.

C and D′ (neutral) = Where they were neutralized before injection.

TABLE 3

The toxicity of hydrochloric acid injected intraperitoneally in guinea-pigs

QUANTITY	1 PER CENT				2 PER CENT				3 PER CENT				4 PER CENT			
	F		R		F		R		F		R		F		R	
	No.	T	No.	T	No.	T	No.	T	No.	T	No.	T	No	T	No.	T
cc.																
0.5	1	42h	1	—												
	1	40h														
1.0	1	10h														
2.0	1	10h														
3.0	1	10h			1 {3h 37m}						1	—	1 {3h 25m}			
4.0	1	10h			1 {4h 24m}				1 {2h 6m}				1 {2h 30m}			
5.0	1	10h			1 {1h 36m}				1 {2h 23m}				1 {1h 12m}			

F = fatalities.

R = recoveries.

No. = number of animals injected.

T = time elapsed between the injection and the outcome.

161

TABLE 4

The toxicity of peptone, proteose, and their derivatives

PREPARATION	HYDRO-CHLORIC ACID CONTENT	NITROGEN CONTENT	DOSAGE PER 350 GRAMS											
			150 mgm.		200 mgm.		300 mgm.		400 mgm.		500 mgm.		600 mgm.	
			F	R	F	R	F	R	F	R	F	R	F	R
	per cent	*per cent*												
W. P.		15.4			0	1	0	1	0	1	0	1	0	1
W. P.—B	8.81	11.72	0	2	1	3								
W. P.—C	8.19	12.24					2	0						
W. P.—D	—	12.24					0	1						
W. P.—D′	—	—							0	1				
DC	—	12.73			0	1					0	1		
DC—A	10.19	11.92					2	0						
DC—B	Neut.	11.92					0	1						
DC—C									0	3				
DC—D									1	0				

W. P. = Witte's peptone.

W. P.-B = acid alcohol extract of Witte's peptone.

W. P.-C = acid alcohol extract of Witte's peptone.

W. P.-D = acid alcohol extract of Witte's peptone neutralized after evaporation.

W. P.-D′ = acid alcohol extract of Witte's peptone neutralized before evaporation.

DC = pure deuterocaseose.

DC-A = acid alcohol extract of deuterocaseose.

DC-B = acid alcohol extract of deuterocaseose neutralized after evaporation.

DC-C = acid alcohol extract of deuterocaseose neutralized before evaporation.

DC-D = same as C to which 40 mgm. HCl has been added.

All of the deaths above recorded are primary with the exception of the one following the injection of DC-D.

F = fatalities.

R = recoveries.

STUDIES ON THE PHYSIOLOGICAL ACTION OF SOME PROTEIN DERIVATIVES

V. THE RELATION OF BLOOD CONCENTRATION TO PEPTONE SHOCK

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An extensive series of recent investigations, notably those of Erlanger and his colleagues, Mann, Bayliss, Dale and his collaborators, has demonstrated that a condition of shock may be accompanied by significant changes in blood volume. The recognition that blood volume changes, especially in the direction of blood concentration, may be of clinical importance in various pathological states is rapidly growing. Thus blood concentration has an undoubted influence on the physiology and pathology of such diverse conditions as cholera (1), war gas poisoning (2), influenza (3), and nutritional disturbances in children (4). In the references cited it is pointed out that blood concentration may play a distinct rôle in the progress of the abnormal condition and may materially influence the ultimate state.

While it is well-known that blood volume changes are bound up with shock and shock-like conditions the details of this relationship have not been extensively investigated. In the present communication such a study has been made, the results of which are outlined below.

METHODS

Full-grown, well nourished dogs were allowed to fast for a period of twenty-four hours previous to the experiment. Anaesthesia was induced by a mixture of morphine (0.01 gram per kilo) and atropine (0.001 per kilo) in the form of the sulphates, and

163

ether. Blood for analysis was drawn from the left femoral artery
and blood pressure was recorded by a mercury manometer
attached to the right carotid artery. Injections were made into
an external jugular vein. Unless otherwise specified in the text
the term "rapid injection" means within a minute. Hemo-
globin estimations were made according to the procedure of
Cohen and Smith; total solid content was determined by drying
blood to constant weight at a temperature of 110°C.

<div style="text-align:center">PEPTONE SHOCK</div>

It has long been known that the intravenous injection of
"Witte Pepton" induces low blood pressure and a shock-like
condition which are accompanied by changes in the rate of blood
coagulation and an accelerated flow of lymph. That these events
may be ascribed to the proteoses themselves and not to adhering
substances, such as histamine, has been advanced by Wolf,
Underhill, Zunz, Gibson and very recently by Hanke and Koessler.
Peptone shock is readily produced and hence affords a simple
means for the investigation of the problem under discussion.

When "Witte Pepton" is injected into dogs under the ex-
perimental conditions outlined above in doses of 0.3 to 0.5 gram
per kilo in a volume of 0.9 per cent sodium chloride solution
approximating 50 cc., a marked fall of pressure results in all
instances, the pressure rarely, if ever returning to the initial level
(see charts 1 to 4 inclusive).[1] Generally the minimal point is
maintained for several minutes and then the pressure slowly
rises to a level approximating 80 mm. Hg., where it may be main-
tained or else there is a secondary fall of pressure culminating in
death. In other experiments the pressure never rises but in-
stead continues to fall slowly but steadily to the zero point.

When the initial fall of pressure is continuous, clotting of the
blood is infinitely delayed. On the other hand if the pressure
returns toward the normal level, clotting time is little delayed.
In other words when injury is not severe enough to cause a
marked effect on blood pressure, clotting is little changed.

[1] The inferences stated in the following pages are drawn from more than 20
experiments with "Witte Pepton."

If hemoglobin content is followed as a measure of changes in blood concentration interesting facts develop which may be best visualized perhaps in the illustrative charts 1 to 4 inclusive.

In the first place evidence is presented of a distinct but variable increase in blood concentration.   From these and other similar experiments it is quite apparent that dosage and rate of injection play a rôle in the development of an increased concentration of the blood.   Thus in chart 1 where 0.3 gram per kilo was injected the rise in concentration was not so great nor so rapid as when 0.5 gram per kilo was introduced in the same period of time, namely, ten seconds.   Moreover, from a comparison of the four charts one may conclude that when the same dosage of peptone is injected at varying rates, the more rapid injection induces a quicker development of the concentration although with the slower injection the ultimate degree of concentration may be greater than with the more rapid introduction of peptone.   On the other hand even with the same dosage and same rate of injection there may be a variable response with respect to blood concentration even though the pressure effects may be almost identical.   It is quite evident therefore that there is an individual variation among the experimental animals relative to resistance to the peptone injection.

In any discussion of the cause for changes in blood concentration in peptone shock at least two factors must be taken under consideration; (a) the influence of the injected substance, (b) the action of the low blood pressure.   It may be assumed that the *direct* cause of blood concentration is the exit of fluid from the capillaries into the surrounding tissues which would account probably for the accelerated lymph flow.   That the fluid exchange is concerned primarily with plasma rather than with water and salts only may be inferred from the fact that parallel determinations of hemoglobin and blood total solids show a distinct relationship but the degree of change is not of the same magnitude, since hemoglobin is more markedly influenced than is the total solid content.

What is the cause for the apparent increased capillary permeability?   Dale and his collaborators have shown that with

histamine there is a capillary damage due to the action of the drug and the fall of pressure induced by histamine is without influence per se. Do these inferences hold with respect to "Witte Pepton?" Can the effects of "Witte Pepton" be ascribed to its content of histamine? The second question can probably be answered in the negative since the quantity of histamine present would undoubtedly be too small to account for the effects produced and also for the reason that according to Hanke and Koessler (6) peptone free from histamine produces typical peptone effects. It is probable that peptone in itself changes the permeability of the capillaries in a manner analogous to that of histamine. This conclusion may be deduced from the following observations. In the first place there does not seem to be a necessary relationship between the level of blood pressure and the duration of low blood pressure, for blood pressure after peptone injection may rather quickly return to above the shock level and yet blood concentration may continue to a high point (see chart 2). Moreover, even though blood pressure may remain at a very low level (see chart 1) blood concentration may not be very greatly increased. Such results would seem to depend more upon the quantity and rate of injection of peptone than upon the level of blood pressure.

From the above considerations it is not intended to convey the impression that a long continued low pressure may not influence the concentration of the blood. It is quite possible that a maintained low pressure may ultimately so interfere with the normal internal respiration of the capillary cells as to induce marked changes in capillary permeability. On the other hand with a pressure maintained at a low level for a short period only, it seems highly improbable that changed permeability induced by inadequate circulation could be an important factor in the initial concentration of the blood observed in peptone intoxication. The change in concentration is very rapid and may continue for a relatively long period after return of the pressure to normal.

If low pressure may be assumed to be the responsible factor in the initial concentration of the blood it would seem quite logical to suppose that hastening the return of pressure to a normal
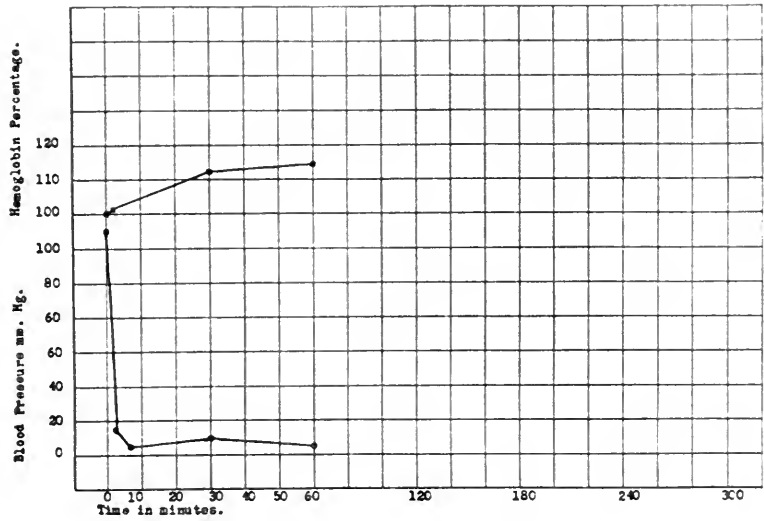
CHART 1.   DOG 1

Injection of 0.3 gram per kilo "Witte Pepton" in ten seconds
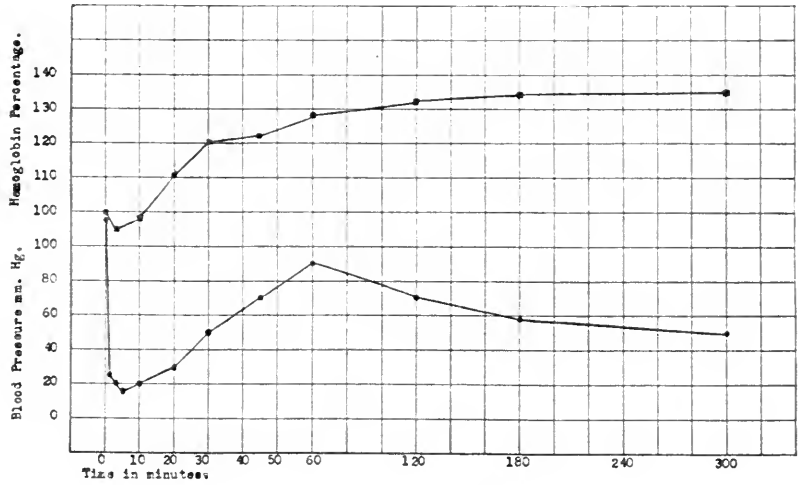
CHART 2.   DOG 48

Injection of 0.5 gram per kilo "Witte Pepton" in ten seconds
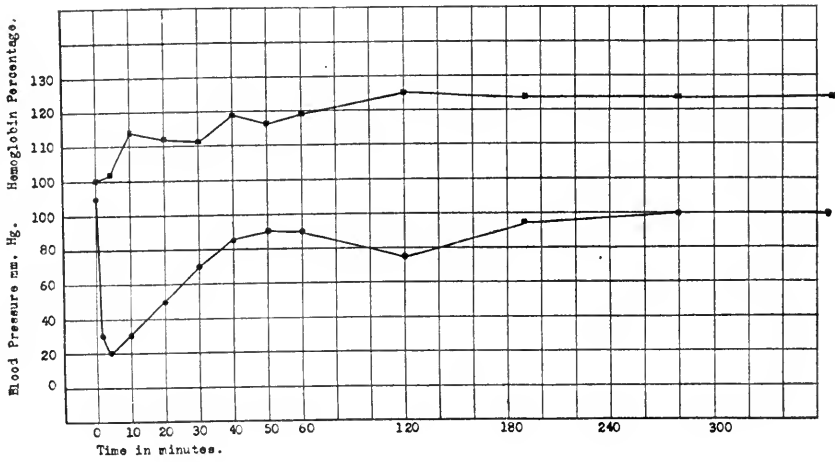
CHART 3.   DOG 46

Injection of 0.5 gram per kilo "Witte Pepton" in two minutes
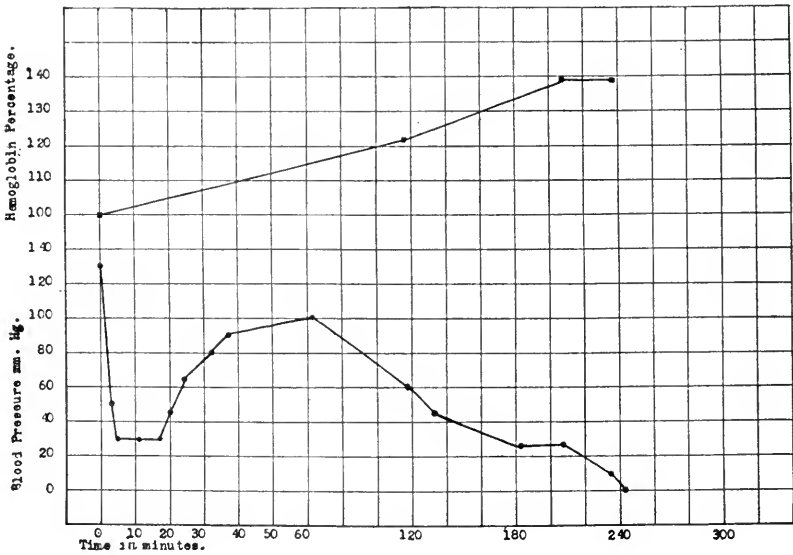
CHART 4.   DOG 7

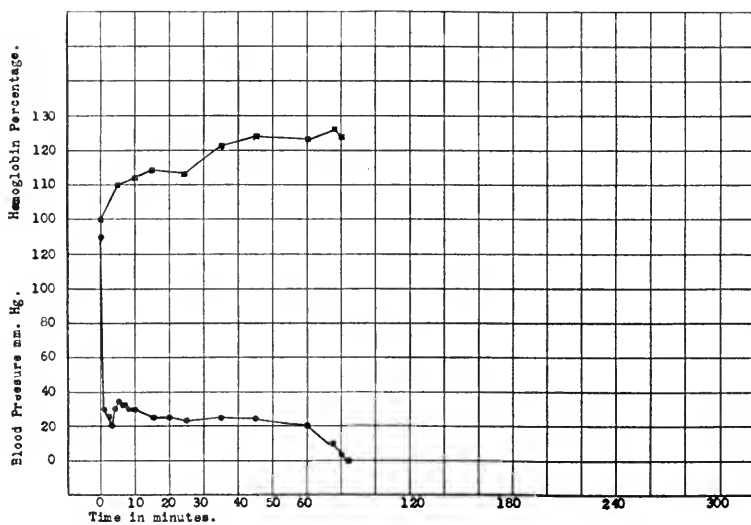Injection of 0.5 gram per kilo "Witte Pepton" in twelve minutes
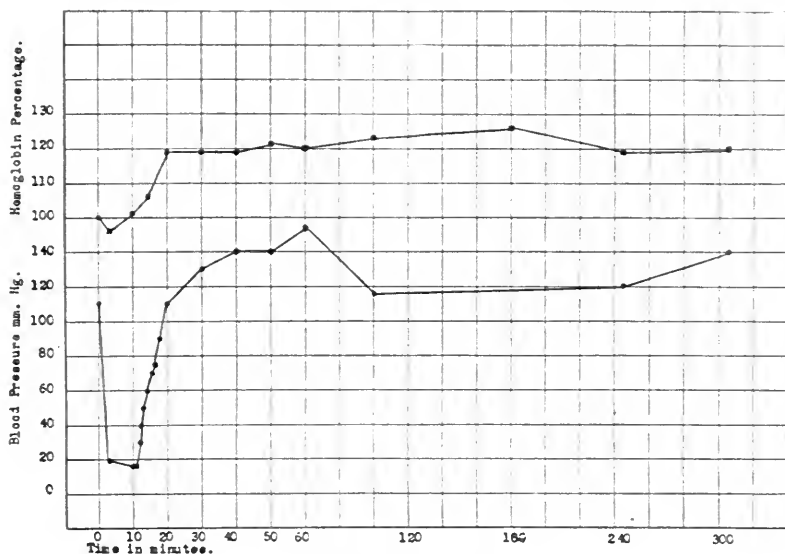
CHART 5.   DOG 51

Rapid injection of 0.5 gram per kilo "Witte Pepton" followed immediately by injection of 6 cc. 1 per cent BaCl₂ solution.
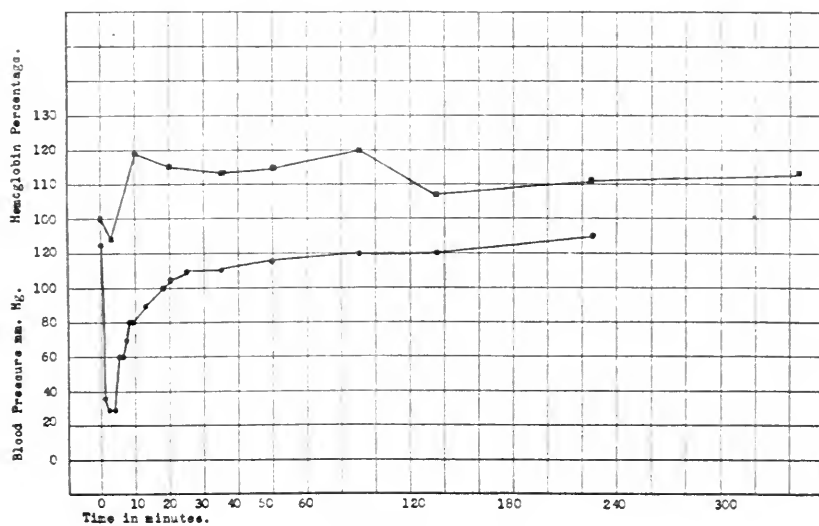

CHART 6.   DOG 52

Rapid injection of 0.5 gram per kilo "Witte Pepton" followed immediately by injection of 6 cc. 1 per cent BaCl₂ solution.
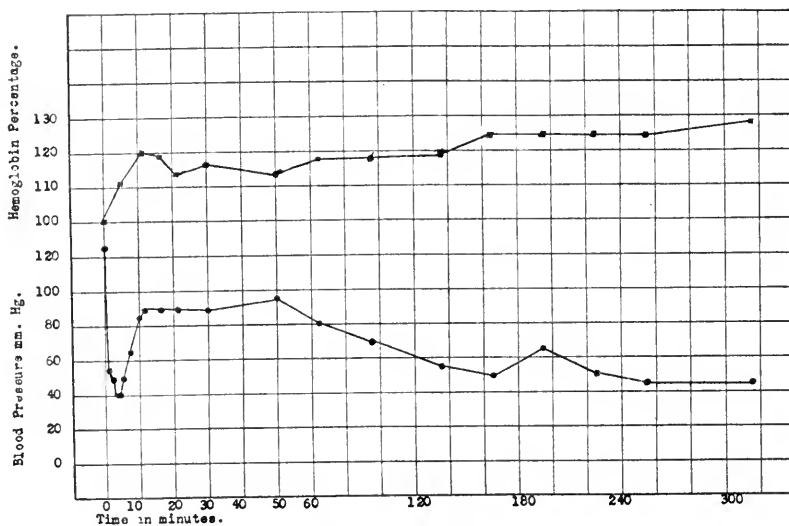
CHART 7. DOG 54

Rapid injection 0.5 gram per kilo "Witte Pepton" followed immediately by injection of 7 cc. 1 per cent $BaCl_2$ solution.
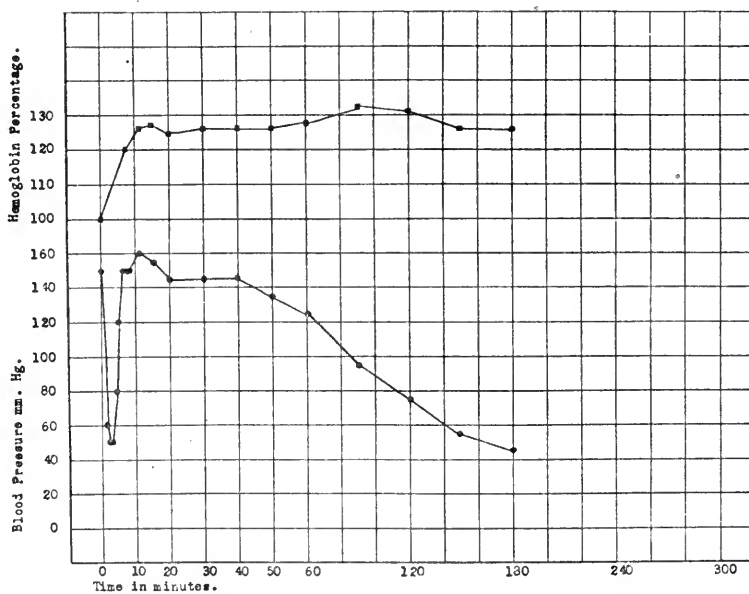
CHART 8. DOG 56

Rapid injection of 0.5 gram per kilo "Witte Pepton" followed immediately by injection of 7 cc. 1 per cent $BaCl_2$ solution.

level would either prevent or modify the initial blood concentration. Attempts to produce only a transitory fall in pressure have been made and the results may be found in charts 5 to 8 inclusive. "Witte Pepton" was injected rapidly and as soon as the pressure had reached the minimal level $BaCl_2$ solution was introduced slowly into a jugular vein. As these examples of many experiments of this sort will show barium chloride was capable of rapidly restoring blood pressure either to the initial normal level or at least well above what may be regarded as a shock level of pressure. From these graphs it is quite evident that the initial rise in concentration could hardly have been produced by the low blood pressure if one assumes that low blood pressure induces changed capillary permeability by interference with internal respiration. On such a hypothesis the interval of low pressure was too brief. One may object to the use of $BaCl_2$ in such an experiment on the ground that $BaCl_2$ acts upon a different set of structures than does the influence exerted by low pressure induced by "Witte Pepton." This fact really however adds strength to our contention since if it is assumed that $BaCl_2$ acts upon the smooth muscle of the arterioles any effect produced initially by the low pressure would probably not be abolished by the $BaCl_2$ and hence the initial damage to the capillaries would not be masked. From the charts it is readily seen that even though the fall of pressure induced by peptone is only transitory after $BaCl_2$ injection the rise in concentration may be just as rapid and great as when the pressure has remained at a low level for a longer period of time. The latter fact is very well illustrated by comparison with chart 9 wherein is recorded an experiment in which $BaCl_2$ failed to raise the pressure after peptone injection.

That low pressure in itself is not the responsible factor in blood concentration may be well seen in chart 10 in which is given an example of the influence of amyl nitrite upon the blood pressure. Here it will be seen that in spite of the fall of pressure, due to a general vaso-dilatation and hence to a decreased blood flow and probable interference with the internal respiration of the capillaries, there is no change in the concentration of the blood.

CHART 9.   DOG 58

Rapid injection of 0.5 gram per kilo "Witte Pepton" followed immediately by 6 cc. 1 per cent BaCl₂ solution.
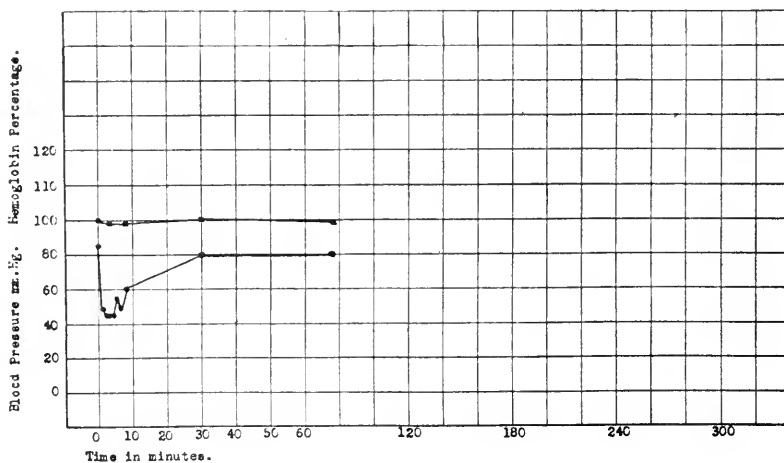
CHART 10.   DOG 61

One-half ounce of amyl nitrite inhaled

From these observations with $BaCl_2$ and with amyl nitrite it seems quite probable that the fall of pressure initially induced by "Witte Pepton" is not responsible for the concentration of the blood observed under the experimental conditions. The only other factor not excluded is the toxic effect of the "Witte Pepton" itself upon the capillary cells. From the fact that a rapid injection of a dose of "Witte Pepton" produces a rapid effect upon blood concentration whereas the same dose introduced in a greater interval of time produces the same degree of concentration but not so rapidly, the pressure in the two instances being practically identical argues for the hypothesis that the quantity of peptone in the circulation at a given period is the effective agency in modifying the development of blood concentration.

Thus far discussion has centered around the initial fall of pressure induced by "Witte Pepton." In many experiments after the first fall the blood pressure may regain a level near the normal or at least above the shock level and then be followed by a secondary fall of pressure. In many of these instances the secondary fall of pressure has been accompanied by a second rise in blood concentration. This is especially noticeable in charts 4, 7 and 8. Here perhaps the failing circulation may be considered quite adequate to explain the relatively slow secondary rise in blood concentration.

Of considerable importance is the question in what measure increased blood concentration is of significance in shock in contributing to a fatal termination. Underhill (2) and Underhill and Ringer (3) have shown that with gas poisoning and in influenza a concentration of blood exceeding 25 per cent of the normal may be regarded as of grave import. When this point is much exceeded death usually follows. It is interesting in this connection to note that in peptone shock a concentration much greater than 25 per cent of the normal is rarely obtained. In other words in this condition the fluid exchange is still within what may be regarded as normal limits, the organism not yet having lost its compensatory powers. It must, therefore, be quite apparent that while concentrations up to this level are

frequently encountered in peptone shock usually the excess is so small as to indicate that blood concentration in itself can hardly be accepted as being entirely responsible for death. It should probably rather be regarded as one important contributing factor.[2]

PURIFIED PROTEOSES

For the sake of completeness and to avoid the possibility that the effects of "Witte Pepton" may be ascribed to contaminating substances rather than to the proteose contained therein experiments have been carried through with purified deutero-proteose.[3]

As an example of the action of this material chart 11 is included showing the influence upon blood pressure and blood concentration. It will be observed that these curves strongly resemble those drawn for similar experiments with "Witte Pepton." It is therefore evident that purified proteoses are quite capable of producing a significant influence upon blood concentration.

[2] On the other hand it is quite possible that the concentration in arterial blood, as determined in these experiments does not represent the true condition of blood concentration in the capillaries, for it has been demonstrated by Cannon, Fraser and Hooper that in shock the red cell count in the capillaries is much greater than in the veins.

[3] The material formed from egg white by pepsin digestion was prepared by saturation with ammonium sulphate after neutralization of the digestion mixture and removal of undigested residue and neutralization precipitate. The proteoses were dissolved in H_2O and dialyzed free from $(NH_4)_2 SO_4$. The mixture was filtered from the small precipitate of heteroproteose and the filtrate evaporated and saturated with NaCl, which precipitated the so-called protoproteose. Treatment of the filtrate from the NaCl saturation with acetic acid precipated a mixture of proto and deuteroproteose. The filtrate from the acetic treatment was dialyzed free from NaCl and constituted the yield of deuteroproteose. The solution freed from NaCl was concentrated to small volume, and treated with alcohol. The precipitate of deuteroproteoses was washed with boiling alcohol and treated with ether while hot, and ground to a fine white powder.
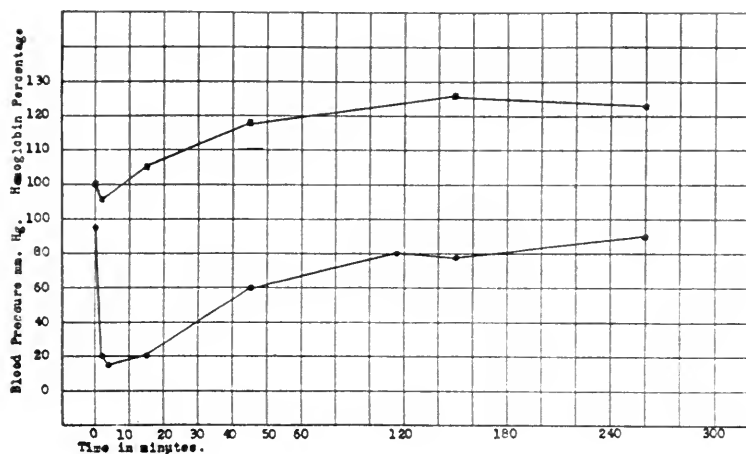
CHART 11.   DOG 34

Rapid injection (five seconds) of 0.5 gram per kilo of deuteroproteoses
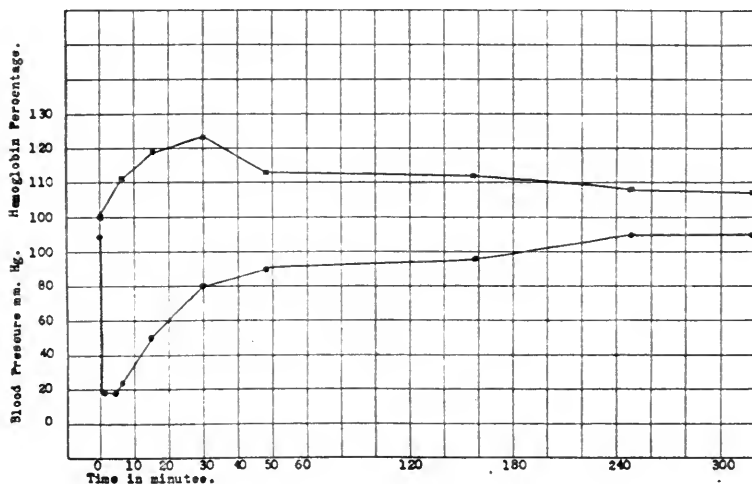
CHART 12.   DOG 40

Injection slowly (in four minutes) 1 mgm. per kilo of histamine
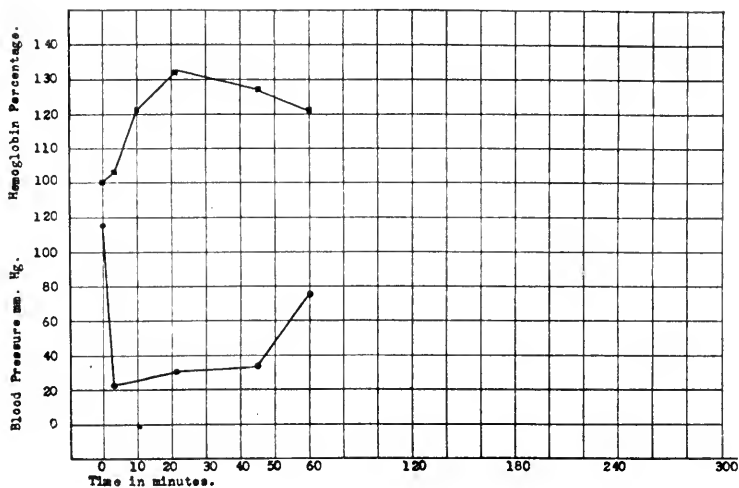
CHART 13.   DOG 16

Injected intermittently during an interval of thirteen minutes 1 mgm. per kilo of histamine.
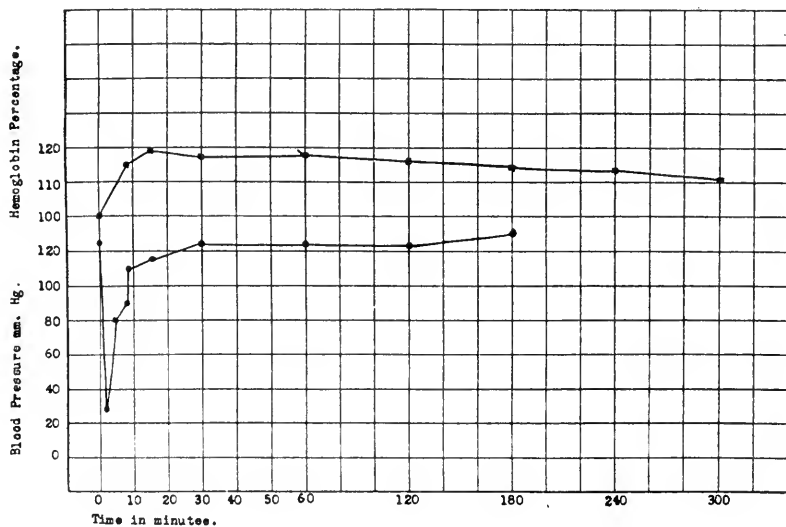
CHART 14.   DOG 53

Injected rapidly 1 mgm. per kilo histamine followed by injection of 7 cc. 1 per cent BaCl₂ solution.

HISTAMINE SHOCK

For comparison with the conditions obtaining in peptone shock a number of observations have been made with histamine hydrochloride. The dosages given are calculated for the base. In charts 12, 13 and 14 are presented illustrative examples of the type of response elicited under varying conditions. Chart 12 shows the influence of histamine injected rather slowly but with a quick return of the pressure to a point above the shock level. Chart 13 illustrates an attempt to keep the pressure below the shock level for a significant period, and in chart 14 are given the results of the action of $BaCl_2$ in preventing low pressure for more than an insignificant period.

These experiments point out quite clearly the marked resemblances between the effects produced by "Witte Pepton" and those evoked by histamine and it is quite apparent that the conclusion drawn above for "Witte Pepton" apply with equal force to histamine. One point of difference, however, seems to be indicated, namely, that the period of high concentration with histamine appears to be shorter than with peptone, that is, there is a decided tendency for blood concentration in histamine shock to quickly descend to the normal level whereas in peptone shock this rarely occurs. May this indicate that the damage to the capillaries in peptone shock is greater than with histamine intoxication, hence resumption of normal conditions is slower?

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(3) UNDERHILL AND RINGER: Jour. Am. Med. Assn., 1920, xxv, 1531.
(4) MARRIOTT: Am. Jour. Dis. Child., 1920, xx, 461.
(5) HANKE AND KOESSLER: Jour. Biol. Chem., 1920, xliii, 567. These authors show that 100 grams of "Witte Pepton" contains the equivalent of 3.35 mgm. histamine hydrochloride.
(6) HANKE AND KOESSLER: Ibid., 1920, xliii, 567.

STUDIES ON THE PHYSIOLOGICAL ACTION OF SOME PROTEIN DERIVATIVES

VI. THE INFLUENCE UPON BLOOD CONCENTRATION OF VAUGHAN'S CRUDE SOLUBLE POISON

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The nature of Vaughan's "Crude Soluble Poison" (1) has been the subject of considerable investigation without yielding definite conclusions. In many respects the material resembles most closely that group of ill-defined substances designated proteoses which undoubtedly consists of a mixture of compounds of varying composition and structure. Like proteose its intravenous introduction produces a profound fall of blood pressure (2) and may infinitely delay clotting of the blood (3) On the other hand, it is considerably more toxic than proteoses both for guinea pigs (4), dogs and rabbits (5). Indeed in their paper Underhill and Hendrix (6) state that "the effects brought about by Vaughan's product must be ascribed to some substance or substances other than the proteoses contained therein since the dosage employed here is far too small to produce any symptoms in the rabbit even though the entire amount of material injected consisted solely of proteoses." Since the above was written we have had occasion to modify the above statement owing to the fact that at the time the above was written it was not realized that Vaughan's product contained such a large content of HCl as was later ascertained. The substances in its dry form may contain as much as ten per cent HCl. Rabbits are extremely sensitive to acid injection and it is quite probable that in the above quoted experiments death resulted as much from acid injection

as from the poison. Rabbits bear the neutralized product in greater doses than the above without a fatal outcome. This apparent important differentiation of Vaughan's crude soluble poison from proteoses is therefore no longer valid.

In a recent communication (7) from this laboratory it is stated that "acid alcohol extracts from Witte's peptone and pure deuterocaseose substances which when evoparated to dryness in acid solution are very toxic, and closely resemble Vaughan's crude soluble poison both in action and potency." The animal employed was the guinea-pig. It would seem therefore that acid alcohol treatment may serve as a means of separating the more toxic from the less toxic substances in the mixture of proteoses. We regard the acid treatment as merely modifying the solubility of certain subtances in the group called proteoses resulting in the separation of the more toxic bodies from those relatively inert. In this sense then Vaughan's crude soluble poison and the acid alcohol extract of proteoses are so far as one may judge substances producing essentially the same physiological response both with respect to kind and degree. It would assuredly be illogical to conclude that these substances are therefore structurally alike. While such a conclusion cannot safely be drawn it must be evident that in respect to general properties and physiological action, the acid extract of proteose and Vaughan's crude soluble poison are very similar.

A recent (8) investigation upon the influence of peptone shock upon blood concentration furnished an opportunity to compare the behavior in this respect of Vaughan's crude soluble poison. Examples of the experiments are detailed below.

METHODS

Full-grown, well nourished dogs were allowed to fast for a period of twenty-four hours previous to the experiment. Anesthesia was induced by a mixture of morphine (0.01 gram per kilo) and atropine (0.001 gram per kilo) in the form of the sulphate and ether. Blood for analysis was drawn from the left femoral artery and blood pressure was recorded by a mercury manometer attached to the right carotid artery. Injections were made into

an external jugular vein.   Unless otherwise specified in the text the term "rapid injection" means injection within a minute. Hemoglobin estimations were made according to the procedure of Cohen and Smith.

The preparation for injection were made by the method of Vaughan from casein.

INTOXICATION BY VAUGHAN'S POISON AND ITS RELATION TO BLOOD CONCENTRATION

In the investigation of the relation of peptone shock to blood concentration it has been demonstrated that Witte's peptone or purified deuteroproteoses are capable of inducing a marked concentration of the blood.   Moreover it is indicated that this change in the blood is probably due to a toxic action upon the capillaries producing an altered permeability whereby plasma makes its exit into the surrounding tissues.

If the intoxication induced by Vaughan's crude soluble poison results in a similar influence upon blood concentration one more resemblance to the proteose-like action would be added to the already long list of resemblances.   It may be stated at once that the introduction into the circulation of the dog of Vaughan's crude soluble poison, in doses of 0.2 to 0.5 gram per kilo, with its acid content neutralized or unneutralized causes the production of a significant increase in the concentration of the blood. The features of this response resemble in every respect those evoked by Witte's peptone or purified proteoses.   It should be stated, however, that the acid product is more toxic than when it has been neutralized, an observation corroborating the conclusions of Vaughan and Pryer and those of Underhill and his collaborators.

From the two typical experiments illustrated in charts 1 and 2 may be observed the influence of Vaughan's crude soluble poison upon blood pressure and blood concentration.   Chart 3 is an example illustrative of the negative influence of Vaughan's non-toxic residue.
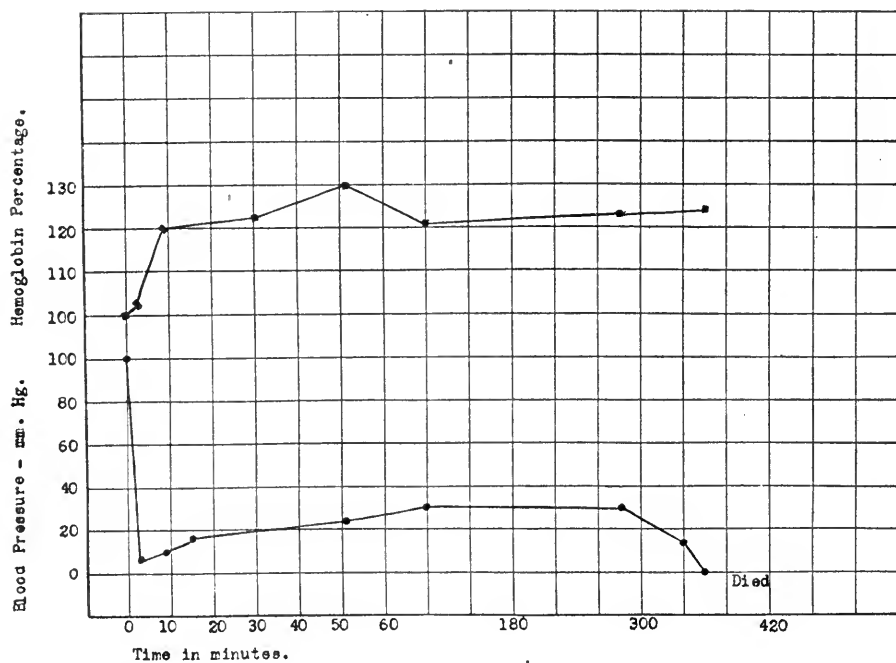
CHART 1.   DOG 19

Rapid injection of Vaughan's crude soluble poison, 0.2 gram per kilo—neutralized, and dissolved in 30 cc. water.
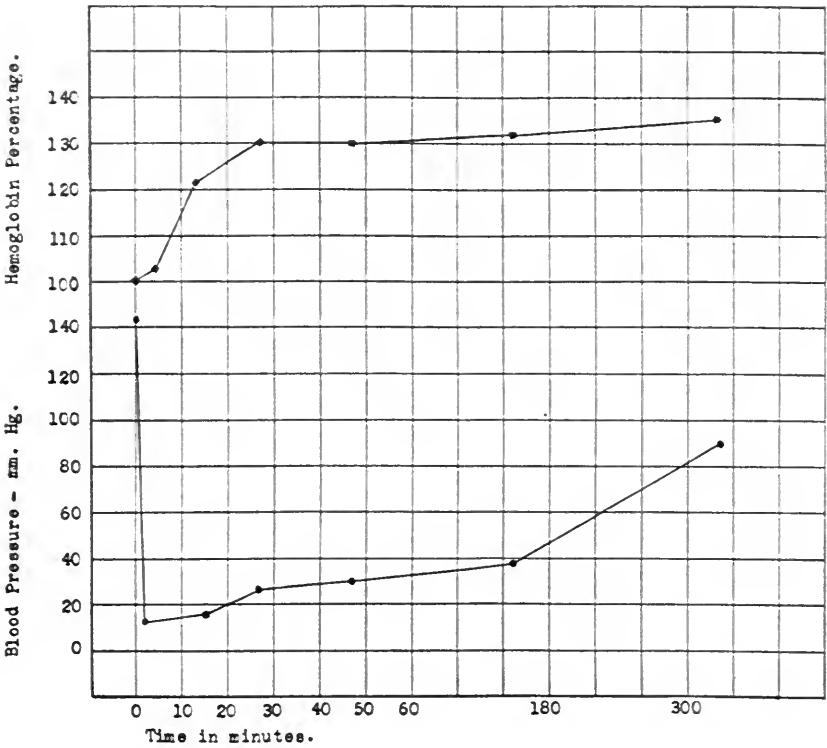
CHART 2.   DOG 20

Rapid injection of Vaughan's crude soluble poison, 0.2 gram per kilo—neutralized, and dissolved in 40 cc. water.

Hemoglobin Percentage.

120
110
100
160
140
120
100
80
60
40
20
0

Blood Pressure – mm. Hg.

0   10   20   30   40   50   60   180   300
Time in minutes.
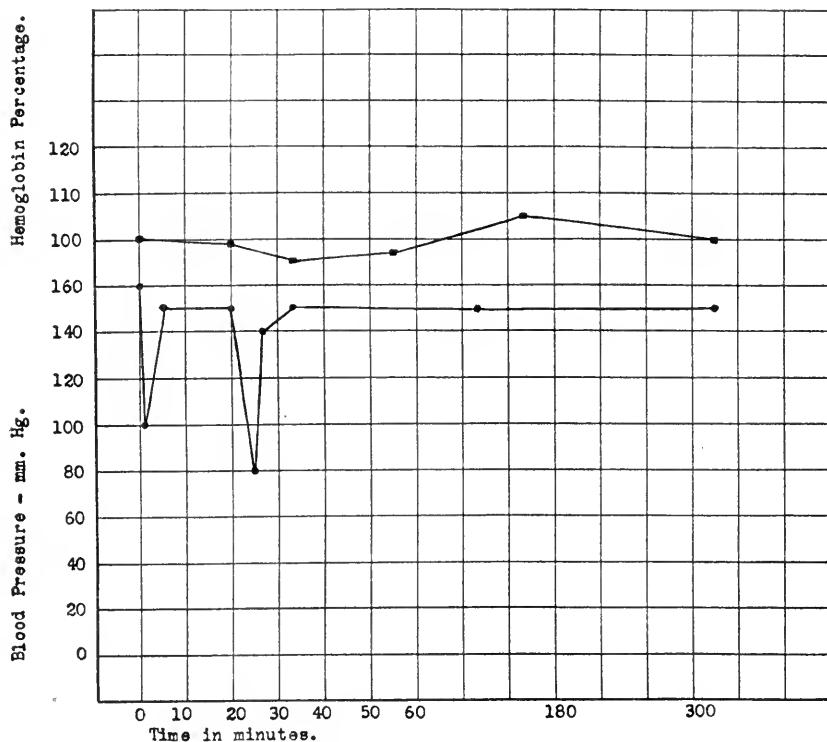
CHART 3.   DOG 22

Rapid injection of Vaughan's non-toxic residue, 0.3 gram per kilo dissolved in 50 cc. water. First curve of fall of pressure caused by injection in three-fourths minutes--second curve of pressure caused by injection of same dose in ten seconds.

CONCLUSIONS

The intravenous injection of Vaughan's crude soluble poison induces a marked concentration of the blood. Vaughan's crude soluble poison and proteoses in similar dosages show striking resemblances in their physiological behavior both producing prolonged fall of arterial pressure, delay in blood clotting and an increase in blood concentration.

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BIOLOGICAL REACTIONS OF ARSPHENAMINE

I. THE MECHANISM OF ITS AGGLUTINATIVE ACTION ON RED BLOOD CELLS IN VITRO

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Since the introduction of arsphenamine into modern therapeutics a vast amount of data has accumulated which indicates the complex nature of the various reactions which occur between it and the tissues and fluids of the body on one hand, and the parasites on the other.

Under the first head come ill-effects, varying from transient vaso-motor disturbances to death, and although certain precautions may be taken to avoid them, nevertheless it is not uncommon for the most severe of them to occur even when all possible care has been taken in the preparation and administration of the drug.

Doubtless much of this uncertainty is due to the fact that our knowledge of the mechanism of these untoward effects is still in the speculative stage, a fact well shown by a consideration of the literature of the subject. It is not our purpose at the present time to enter into a discussion of these various theories. Suffice it to say that the accidents have been explained by factors either chemical in nature, (Schamberg et al. (1) physical, (Fleig (2), Danysz (3)) or by what one might call humoral (Berman (4)).

In our studies we have found certain physical factors which we believe explain some of the ill effects following arsphenamine administration. Among the most important of these is that of agglutination of the red blood cells by the drug.

That arsphenamine produces an agglutination of red cells in vitro has been shown by Karsner and Hanzlik (5). They also demonstrated that this property is shown by various colloids which are sometimes administered intravenously, such as acacia and gelatin, and pointed out that the anaphalactoid reactions which often follow such use, are not analogous in their mechanism to anaphalaxis, but are most likely the result of agglutination in vivo.

It has been known for some time that many substances in a colloid state produce agglutination of red cells in vitro Among those who have studied this phenomonon may be mentioned Landsteiner and v. Jagic (6), Gengou (7), and Henri and Girard-Mangin (8). A discussion of their findings is reserved until our results are given.

GENERAL DESCRIPTION OF THE PHENOMONON OF AGGLUTINATION

In all the experiments described the disodium salt of arsphenamine was used. The stock solution was prepared in 2 per cent solution in double distilled water, by the standard method of the United States Hygienic Laboratory in which 0.9 cc. of $\frac{N}{1}$ NaOH are added to each 0.1 gram of the drug. The solution was prepared fresh for each experiment and used at once

Rabbit cells were used in most of the experiments. They were drawn in isotonic sodium citrate, washed three times with 0.9 NaCl, and made up to 5 per cent suspension in the same fluid. Incubation was done in a water bath at 37°C.

The titre of arsphenamine solution

For the titration of arsephenamine solution, $\frac{1}{4}$ cc. of 5 per cent cell suspension, $\frac{1}{4}$ cc. of 0.9 NaCl and $\frac{1}{4}$ cc. of various dilutions of arsphenamine were incubated together for two hours at 37°.

A fairly constant titre was observed with different preparations of arsphenamine and cells. The greatest dilution which caused complete agglutination in the majority of cases was $\frac{1}{64}$

(0.011 per cent[1]). The dilution at which no agglutination could be made out varied around $\frac{1}{256}$ (0.002 per cent).

Some variation was noted in the agglutinability of cells of different species, as reference to table 1 will show. Human cells are most strongly acted upon, while those of the chicken are least affected.

TABLE 1

Titre of agglutination in various species

BLOOD CELLS	ARSPHENAMINE DILUTIONS										
	C	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	C†
Human................	H	H	C*	C*	C*	C	C	C	xx	O	O
Rat..................	H	C*	C*	C*	C*	C	C	xx	x−	O	O
Guinea-pig..........	H	H	C*	C*	C	C	C−	xx	O	O	O
Ox..................	H	C*	C*	C	C	C	C	x	O	O	O
Rabbit..............	H	C*	C*	C	C	C	C	x−	O	O	O
Frog................	C*	C*	C*	C	C	C	C	x−	O	O	O
Sheep...............	H	H	C*	C*	C	C·	x	O	O	O	O
Chicken.............	H	H	C*	C*	C	C	x	O	O	O	O

* Some hemolysis.
† Control of salt solution and cells.

The occurrence of a "proagglutinoid zone"

If the occurrence of agglutination in a series of dilutions is observed during the progress of the reaction, a marked difference is noted in the time of appearance of clumping and also the time required for the completion of the process. In the majority of cases the agglutination is first observed in the $\frac{1}{32}$ dilution, (0.022 per cent), and is as a rule complete before any change can be noted in the $\frac{1}{16}$ dilution (0.045 per cent). After the $\frac{1}{16}$ dilution, the $\frac{1}{8}$ (0.09 per cent) and $\frac{1}{4}$ (0.18 per cent) follow, while hemolysis obscures the readings in the higher concentrations. Dilutions weaker than $\frac{1}{32}$ vary in the time of appearance of agglutination though it is not definitely visible until the changes in the stronger ones are complete.

[1] The percentages given for the various dilutions represent the concentration of arsphenamine in the agglutination tube after the addition of cell suspension and salt solution.

The effect of standing on dilutions of arsphenamine

If the stock tubes containing the arsphenamine dilutions in NaCl are allowed to stand uncorked at room temperature, there is a marked change in their physical appearance. After a few minutes, varying from five to twenty, a slight turbidity occurs first in the $\frac{1}{32}$ dilution and then in the $\frac{1}{16}$, $\frac{1}{8}$, and $\frac{1}{4}$ in the order named. Still later a similar change is noted in the $\frac{1}{64}$. After several hours the turbidity becomes a flocculent precipitate, which is easily broken up into a homogenous suspension

TABLE 2

Effect of standing on titre of arsphenamine dilutions

TIME OF TEST AFTER PREPARATION OF ARSPHENAMINE DILUTIONS	ARSPHENAMINE DILUTIONS								PHYSICAL STATE OF DILUTIONS
	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	
At once..................	H	H	C*	C	C	C	x	x	All clear
2 hours..................	H	C*	C*	C−	C−	x	O	O	$\frac{1}{16}$ to $\frac{1}{32}$ turbid
6 hours..................	C*	C*	C	C−	x	O	O	O	$\frac{1}{4}$ to $\frac{1}{128}$ turbid
22 hours..................	C*	C	C−	x	O	O	O	O	All densely turbid
22 hours, clear supernatant	O*	O	O	O	O	O	O	O	No further change

* Some hemolysis.

by shaking. The color of the suspension also begins to darken. After twenty-four hours the tubes are filled with a dense brownish precipitate, the amount varying with the concentration of the dilution.

As will be demonstrated later, there are two processes at play in this change, one depending on the action of the NaCl and absorbed CO_2 with the arsphenamine, and the other on the oxidation of the later. At present we are not so much interested in the changes in the solution as in the resulting differences noticed in its power to agglutinate red cells. This suffers a steady drop which may be followed for twenty-four

hours, at which time the change in the titre is as a rule complete. Table 1 shows the change in titre after 2, 4, 6, and 24 hours. Before distributing the cloudy dilutions to the agglutination tubes they were thoroughly shaken so that an even suspension of arsphenamine mixture was added. At the end of the experiment the precipitate was removed from the dilutions by centrifugalization and the clear supernatant titrated. No agglutination was observed, although hemolysis still occurred in the stronger dilutions. No further precipitate occurred in the cleared dilutions in the course of a week.

With the drop in the titre of the altered arsphenamine there occurred a corresponding "shift to the left" in the pro-zone. Whereas in the fresh preparations whose weakest effective dilution was $\frac{1}{256}$ (0.002 per cent), the optimum dilution was $\frac{1}{32}$, when the weakest effective dilution had dropped to $\frac{1}{32}$, the optimum dilution was $\frac{1}{4}$.

THE RÔLE OF THE ELECTROLYTE IN THE PRODUCTION OF AGGLUTINATION

To determine if a certain amount of electrolyte was necessary for the occurrence of agglutination the following experiments were performed. The red cells were washed in isotonic sugar, 6 per cent saccharose, until testing with $AgNO_3$ showed the wash fluid to be practically free of chlorids. Spontaneous agglutination of the cells may occur, especially after they have been washed several times. This is most likely due to an absorption of CO_2 from the air as suggested by Coulter (9). We found it less likely to occur if 6 per cent. sugar was used instead of the stronger 10, and had no difficulty with hemolysis from hypotonicity. The danger of spontaneous agglutination was also met by suitable controls.

If $\frac{1}{4}$ cc. of this sugar suspension of cells is added to the optimal agglutinating dose of arsphemamine, $\frac{1}{4}$ cc. of $\frac{1}{32}$ dilution, and a final $\frac{1}{4}$ cc. of sugar solution added, no agglutination occurs on incubation for two hours. A control of $\frac{1}{2}$ cc. of sugar solution and $\frac{1}{4}$ cc. of cells serves as a control against spontaneous agglutination, and also shows no agglutination.

If, however, NaCl is added either in the form of concentrated solution or a small crystal, in either case attempting to avoid unnecessary dilution, prompt agglutination occurs in the tube containing arsphenamine, and none in the other.

Stronger concentrations of arsphenamine have no more effect in producing agglutination in the absence of electrolytes as is shown in a complete titration, using sugar as a dilutent of the arsphenamine and a sugar suspension of cells. As may be seen

TABLE 3

Titration of arsphenamine in absence of electrolyte

				ARSPHENAMINE DILUTIONS IN SUGAR SOLUTION							
	C	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	C*
Agglutination..............	—	—	O	O	O	O	O	O	O	O	O
Hemolysis.................	C	C	xx	x	tr.	O	O	O	O	O	O

* Control of sugar solution and cells.

TABLE 4

Effect of various ions in producing agglutination

$\frac{1}{4}$ *cc. each of 5 per cent red cells in sugar solution, $\frac{1}{32}$ arsphenamine in sugar solution, and sugar solution. Incubated one hour at 37. No agglutination. 3 drops of dilutions of molar solutions of electrolyte added, incubated 2 hours.*

	CONCENTRATION OF ELECTROLYTE SOLUTION ADDED								
	4M	2M	M	M/2	M/4	M/8	M/16	M/32	M/64
NaCl...............	xx	x	x—	O	O	O	O	O	O
KCl................	xx	xx	x	x—	O	O	O	O	O
BaCl₂.............			C	C	C	C	C	xx	O
CaCl₂.............			C	C	C	C	C	C	x—

from table 3 no agglutination occurs even in the tube containing concentrated arsphenamine. It is also interesting that hemolysis occurs to as great a degree as was observed in the previous titration whose tubes contained NaCl.

Other electrolytes produce the same agglutinating effect on cells previously incubated with arsphenamine in sugar solution. A rough test of the efficiency of other ions was made in the following manner. A number of tubes containing sugar solution, sugar arsphenamine and sugar cells in the usual proportions were

incubated for one hour at 37°. They were then arranged in series and 3 drops of different dilutions of molar solutions of various salts added. Table 4 indicates the result. From these findings an ion series may be constructed of the following type, Na < K < Ba < Ca.

THE ABSORPTION OF ARSPHENAMINE BY RED BLOOD CELLS

The experiments just detailed have shown that prolonged incubation of red cells and arsphenamine in the absence of a certain amount of electrolyte results in no agglutination. That there is an intimate union between the cells and the arsphenamine, however, is shown by the following experiment.

A 5 per cent suspension of sugar cells, sugar arsphenamine, and sugar solution in the usual proportions were incubated for one hour at 37°. The tube was then centrifuged, the supernatant fluid removed and the cells washed four times with a great excess of isotonic sugar. Six hundred times the bulk of the cells was used in all. After washing, a proper amount of sugar solution was added to bring the cells to the original 5 per cent suspension. This suspension showed no agglutination on incubation. The addition of NaCl or other electrolyte in concentrated form as described above, produced complete agglutination. As a rule there was some delay in the occurrence of the reaction, but in all cases it was complete in one half hour.

THE MECHANISM OF THE ELECTROLYTE ACTION

If a 2 per cent solution of arsphenamine is diluted with double distilled water, great care being taken that all the glass ware is perfectly clean, the dilutions are water clear and remain so for several hours. Occasionally a slight turbidity develops in the $\frac{1}{32}$ dilution. The same clear dilutions may be prepared with isotonic sugar solution, and they will remain clear for even several days.

If, however, 0.9 NaCl is used as the dilutent, in a few minutes the physical state of the diluted fluids changes. These changes have been detailed in a previous section dealing with the drop

in titre observed in such solutions as they stand. It consists in the development of turbidity and precipitation, first in the $\frac{1}{32}$ dilution and later in the more concentrated ones, the $\frac{1}{2}$ dilution remaining clear as a rule for an hour.

The absolute speed of the process in the various experiments varied, but the relative rate between the various dilutions was constant. The concentration of the salt plays a rôle in this regard, since, if 9 per cent NaCl is added, even the $\frac{1}{2}$ dilution becomes immediately turbid.

A further point regarding the reaction may be mentioned at this place. A very small amount of either alkali or acid will clear the turbid fluids. Preliminary determinations have shown that the pH range at which turbidity occurs is from 3.3 to 9.6.

In the present paper we shall not study this phenomenon further, as it is our desire to discuss here only the general application of it to the process of agglutination. As a working hypothesis we may assume that within a certain range of acidity, reached in our experiments partly by dilution but even more so by absorption of CO_2 from the air, the electrolyte and the CO_2 cause a change in the physical state of the solutions by action on the arsphenamine. These changes vary from a slight Tyndal phenomenon when the solutions are tested with a light beam, to a dense precipitate, depending on the concentration of the various reagents, in particular the electrolyte. It is highly likely that in the later stages the process is complicated by oxidation of the arsphenamine.

As the electrolyte shows this effect in these experiments, and as all the conditions for similar reactions are present when agglutination occurs, and as agglutination does not occur when these conditions do not obtain, it would seem that the action of the electrolyte in the process of agglutination is on the arsphenamine molecule. Further evidence is found in the analogous "prozones" which occur in the same order and dilutions in the two reactions.

It is our purpose to report later in some detail on the part played by the H ion concentration and the action of ions of vari-

ous valency, together with the influence of the former on the adsorption of arsphenamine by the red cells.

DISCUSSION

In reviewing the literature of agglutination of red cells by chemical substances, we find that in the great majority of instances these substances are active in the colloidal state. Such for instance are the studies of Landsteiner and v. Jagic (6) on various inorganic acids and bases, those of Gengou (7), on calcium fluoride, and Henri and Girard-Mangin (8) with ferrous hydrate. It is therefore of interest to determine if arsphenamine may be included in this group.

Properly alkalinized di-sodium arsphenamine has the general appearance of a true solution. But Fleig (2) has pointed out the different reactions which may occur under certain conditions. He noticed that the physical state of the solutions, in particular their clearness, varied with the amount of NaOH and NaCl present. An insufficient amount of the former with an excess of the latter produced a distinct turbidity. This he explained as being due to a precipitation of the arsphenemine base. Theoretical equations are given by him for this double reaction. Danysz (3) has elaborated these ideas by showing that many substances cause a precipitate in arsphenamine solutions. NaCl and CO_2 are included among these substances.

Other writers have called attention to the possibilities of changes in the physical state of arsphenamine solutions without giving details as to the reactions. The English Salvarsan Committee (10) suggests that the ill-effects sometimes seen following salvarsan are due to them. Dale (11) points out that salvarsan prepared in alkaline solution is practically insoluble at the pH of the blood, and must therefore circulate in a colloidal form, prevented from aggregation by the plasma proteins. Karsner (5) and Hunt (12) also suggest that the physical properties of the solutions may be of importance in producing its toxicity.

As stated in the body of this paper we reserve a detailed discussion of the mechanism of the reaction produced by the

addition of electrolyte on arsphenamine solutions for a further study. We do wish to emphasize, however, that in the absence of a certain amount of electrolyte, in sugar solution for instance, no observable change occurs in the physical state of the solution, while the addition of electrolyte, particularly to dilute solutions, causes such a change, varying from a slight Tyndal phenomenon to a flocculent precipitate.

Our experiments have shown that in the former case, i.e., in sugar solutions, no agglutination of red cells occurs, while in the presence of a certain amount of electrolyte, agglutination is prompt. Arsphenamine therefore, may be included among those substances mentioned previously, which by virtue of their physical properties cause agglutination.

Although we have made no special study of the hemolysis caused by arsphenamine it is evident from certain experiments that the mechanism of its production must be quite different. It occurs equally well in the absence of electrolyte as when salts are present and there is only a slight drop in the degree of its production as the dilutions stand, i.e., during the precipitation of arsphenamine. The old clear solutions after the process of precipitation was completed, produced marked hemolysis, but no agglutination.

In our experiments on the effect of standing on the agglutinating titre of salt dilutions, we have seen that as the reaction of precipitation proceeds, there is a gradual loss in the agglutinating power of the solution. Fully precipitated solutions which have been cleared by centrifugalization are no longer potent even in high concentration. These two facts suggest that it is not so much the actual physical state of the solution which is responsible for agglutination as it is either, the process of that change of state from a widely dispersed condition to one of larger visible aggregates, or that it is due to the site of this reaction. For if it were not the reaction itself, rather than the result of the reaction, i.e., the precipitate, those solutions which had stood 4 hours and which were distinctly turbid, would be more efficient in producing agglutination than the clear ones which had just been prepared.

This brings us to a consideration of the mechanism of the process of agglutination. As we have shown in our experiments, the red cells "bind," or perhaps better, adsorb arsphenamine so firmly that repeated washings will not remove it. Cells in sugar may be sensitized, and latter agglutinated with electrolyte. Further evidence of this reaction is seen in the brownish discoloration of the hemoglobin of the cells which results in the presence of arsphenamine. With this knowledge, and remembering the action of electrolyte on arsphenamine, one may assume that it is the reaction between the red cell-arsphenamine complex and electrolyte, resulting in a product analogous to a precipitate, that causes a clumping of the cells. The actual clumping may be due to a mechanical glueing together of the cells, for the reaction would occur at the contact surface of the arsphenamine saturated cells and the electrolyte solution, or perhaps to changes in the electric charges of the cells during the reaction.

The former suggestion corresponds very closely with the theory advanced by Henri and Girard-Magnin, who consider the electrolyte action as occurring in the peri-globular zone.

CONCLUSIONS

1. Arsphenamine has a fairly constant agglutinating titre for red blood cells.

2. The cells of different species vary somewhat in their agglutinability. Human cells are most strongly acted upon, chicken cells the least.

3. There is a drop in the titre of salt dilutions of arsphenamine as they stand in the open air.

4. Arsphenamine is absorbed by red cells, but no agglutination occurs except in the presence of electrolyte.

5. A physical change in the degree of dispersion of arsphenamine results when electrolyte is added to arsphenamine in solution.

6. It is suggested that the action of electrolyte in the process of agglutination is due to this action on the adsorbed arsphenamine of the "sensitized" cells.

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BIOLOGICAL REACTIONS OF ARSPHENAMINE

II. THE PROTECTIVE ACTION OF HYDROPHILIC COLLOIDS ON THE AGGLUTINATION OF RED BLOOD CELLS BY ARSPHENAMINE

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In a previous article the mechanism of arsphenamine agglutination has been studied (1). It was shown that the arsphenamine was "bound" by the red cells but that in the absence of a sufficient amount of electrolyte no agglutination occurred. If this was added prompt agglutination resulted. Evidence was presented tending to show that the action of the electrolyte was on the arsphenamine absorbed by the cells, as it produced a definite change in the physical state, dispersion, of this substance when studied under similar conditions.

At present we wish to study the inhibitive effect of certain hydrophilic colloids on this process, and analyse, if possible, its mechanism. It has been known for some time that agglutination by various chemical substances may be prevented by the presence of certain of these colloids. That blood serum, if present in very small amounts will prevent the agglutination of red cells by Ba_2SO_4 was shown by Gengou (2). Landsteiner and v. Jagic (3) and Henri and Girard-Mangin (4) also demonstrated the same phenomenon in agglutination by several colloidal substances.

In agglutination of red cells by arsphenamine we have the advantage of working with a definite chemical substance, the method of whose agglutinating action has been analyzed, so that it is of particular interest to study the converse protective action of hydrophilic colloids.

199

THE COMPARATIVE EFFICIENCY OF VARIOUS COLLOIDS IN THE PREVENTION OF AGGLUTINATION

In these experiments a 5 per cent suspension of thrice washed rabbit cells in 0.9 NaCl was used. The 2 per cent disodium arsphenamine was also diluted with 0.9 NaCl and a $\frac{1}{32}$ dilution

TABLE 1

Protection from agglutination by various colloids

	COLLOID DILUTIONS								
	C	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	C¹	C²
Starch, 4 per cent.....................	H	O	O	x	C	C	C	O	C
Egg albumin, 4 per cent...............	O	O	x—	x	C	C	C	O	C
Gum arabic, 3 per cent................	O	O	O	O	x	x	x	O	C
Gelatin, 3 per cent....................	H	O	O	O	O	x	x	O	C
Serum................................	O	O	O	O	O	x—	x	O	C

Each tube contains: $\frac{1}{4}$ ccm. dilution of colloid, $\frac{1}{4}$ cc. $\frac{1}{32}$ arsphenamine dilution, $\frac{1}{4}$ cc. 5 per cent red cell suspension.

C¹ contains colloid, cells and salt.

C² contains arsphenamine, cells and salt.

TABLE 2

Summary of protective experiments given in form of gold number

COLLOID	CONCENTRA-TION USED	AMOUNT PREVENTING AGGLUTINA-TION	RECIPROCAL	RELATIVE VALUE OF RECIPROCAL
	per cent	*gram*		
Gelatin.....................	3	0.00017	5882	8.5
Serum......................	7*	0.00025	4000	5.8
Gum arabic.................	3	0.00035	2857	4.1
Egg albumin................	4	0.0006	1666	2.4
Starch.....................	4	0.00145	689	1.0

* Per cent protein in blood serum.

(0.022 per cent) used, as previous experience had shown that this is the optimal agglutinating strength. To a series of tubes containing $\frac{1}{4}$ cc. of the two above constituents, $\frac{1}{4}$ cc. of progressive dilutions of the colloid studied were added, and the tubes incubated for two hours at 37°. In each case a control consisting of

$\frac{1}{4}$ cc. each of cells, NaCl solution and the $\frac{1}{4}$ dilution of the colloid was prepared, as it is known that many of these substances will themselves agglutinate red cells at certain concentrations. The substances studied include, fresh blood serum and aqueous solutions of gelatin, gum arabic, starch, and Merk's egg albumin.

Table 1 shows the results obtained in a characteristic experiment. In experiments done with solutions of the colloids prepared at different times, there was some difference in the readings of the weaker dilutions, as it was often difficult to determine whether or not slight agglutination had occurred.

Table 2 shows the various colloids arranged in the order of their relative efficiency as determined in a series of experiments. It is based on the grams of the substance which just failed to prevent agglutination, with the reciprocals of these quantities and the relative values of the latter.

THE EFFECT OF HYDROPHILIC COLLOIDS ON THE ADSORPTION OF ARSPHENAMINE BY RED CELLS

It has been previously shown that one phase of the process of agglutination of red cells by arsphenamine consists in an adsorption of the arsphenamine by the red cells. The following experiments were performed to see if those colloids which prevent agglutination affect this part of the process.

One cc. of 5 per cent rabbit cells in isotonic sugar was incubated for one hour with 1 cc. each of rabbit serum and the optimal agglutinating dilution of arsphenamine, i.e., $\frac{1}{32}$ (0.022 per cent). A control of equal amounts of sugar cells, sugar solution and arsphenamine was also prepared. No agglutination occurred in either tube. The cells in both were then separated by centrifugalization, washed several times with an excess of sugar solution and finally made up to the original 3 cc. with sugar solution. The suspension in both tubes showed no agglutination on incubation for twenty minutes. A sufficient amount of electrolyte solution, $CaCl_2$, was added and the tubes reincubated. The sugar control, in which there had been no serum, showed complete agglutination. The tube which had previously contained

serum on the other hand, showed no agglutination whatever, demonstrating that the serum had prevented the "binding" of the arsphenamine by the cells.

Further evidence that the serum prevents the union of the cells and the arsphenamine is shown by the contrast in the color of the tube containing serum and arsphenamine and that containing arsphenamine alone. Towards the end of the first incubation, the cells in the control serum-free tube are a dull brown from the action of the arsphenamine on their hemoglobin, while the cells in the tube containing serum are the original bright red.

THE EFFECT OF HYDROPHILIC COLLOIDS ON THE REACTION BETWEEN ARSPHENAMINE AND SALT

The second phase of the process of agglutination has been shown to consist of an action by the salt on the arsphenamine. It is therefore necessary to determine if this part of the reaction is also affected by the presence of a protective colloid. From the well known action of these substances on the flocculation of suspension colloids by electrolyte one would expect such an effect. The point was investigated in the following manner.

A 2 per cent solution of arsphenamine was diluted with a mixture of equal parts of 0.9 NaCl and serum. Another similar series was diluted with 0.9 NaCl alone. Observation of the series showed that while in the series that contained no serum, the physical state of the dilutions changed from their perfectly clear state to varying degrees of turbidity and precipitation, depending on the concentration of arsphenamine, in that series in which serum was present, no such change occurred. The reaction between arsphenamine and salt is therefore inhibited by the presence of serum.

DISCUSSION

Our experiments have shown that many hydrophilic colloids exert their usual protective action when in the presence of arsphenamine and red cells. Moreover the degree of their efficiency in preventing agglutination corresponds roughly to the

degree of protection they afford to suspension colloids from flocculation by electrolyte.

Table 2 is based on Zsigmondy's method of determining the "gold number" of these hydrophilic colloids. In this method the number of milligrams of a substance which just fails to protect a standard gold solution from color change by electrolyte is determined and expressed as a reciprocal for comparison. Zsigmondy found that three classes of protective substances could be established, depending on the degree of their protection; in class I were glues and gelatin, class II egg albumin and gum arabic, and in class III, starches. Reference to our table shows that the same classification holds good for their protective power against agglutination. One discrepancy inside class II was noted, in that gum arabic was more protective than Merk's egg albumin, the reverse of Zsigmondy's findings. He however noted wide variations in the value of different samples of gum arabic, and this fact combined with the difficulty in determining small amounts of agglutination makes an exact quantitative comparison of our results with his impossible.

It is also evident from the experiments detailed above that the inhibitive effect of certain colloids on the agglutination of red cells by arsphenamine is due to factors exerted on both phases into which previous studies have shown that the process may be divided. These phases consist, first in an adsorption of the arsphenamine by the red cells, and secondly, a change in the physical state of the arsphenamine by the action of the electrolyte ions present. The inhibition of both these reactions may be observed separately in proper experiments, and such observations, will explain the lack of the combined process with resulting agglutination, which is found when the same conditions are present in the agglutination experiments.

The exact manner in which the serum (colloid) inhibits these reactions is not at present open to any very obvious method of experimental study. We find in the literature different suggestions for the explanation of analogous inhibitive reactions with protective colloids. Bechold (6) and Neisser and Freidmann (7) have attributed the prevention of precipitation of

suspensions and bacteria by protective colloids to a homogenous encircling of the suspended particles by the protecting substance, so that the precipitating reagent cannot come in contact with them.   Billitzer (8) however, concludes that there is no union between the protective and protected substances, but rather that the former adsorbs the precipitating electrolyte thus preventing its action.   Zsigmondy has suggested that the protective action is due to a union (adsorption) of several particles of the protective colloid with a single larger particle of the suspended material.

Gengou (2) in his studies on the agglutination of red cells by Ba_2SO_4 explains the inhibitive effect of serum in the following way. Ba_2SO_4 causes agglutination by its adsorption to the red cells.   The action is direct, salts playing little if any rôle in the process.   The process is prevented if "stable" colloids (serum, gum arabic) are present, by the "adhesion" of these substances with the agglutinating agent.   The inhibition is therefore due to the substitution of one set of adsorptive phenomena (serum Ba_2SO_4) for the other (red cell-Ba_2SO_4).   He compares the process with the stabilization of a suspension of Ba_2SO_4, for in the last analysis he considers the agglutination of cells by this substance as an exaggeration of the normal tendency of this heavy salt to sediment.

Henri and Girard-Mangin (4) also noted the inhibitive action of serum on the agglutination of red cells by various colloidal substances.   This agglutination they considered due to the precipitation of the colloidal substance by electrolyte.   The latter is derived from the red cells by the diffusion from them of their contained salts so that each cell is surrounded by a zone of high salt concentration.   In this zone flocculation of the colloidal substance occurs and by means of this precipitate the cells are bound together in clumps.   Hydrophilic colloids prevent the agglutination by preventing the flocculation of the unstable colloid by the diffused electrolyte.

In applying these suggestions to our experiments a primary difference is noted, in that the agglutination of red cells by arsphenamine is a double process, and that the prevention of

this agglutination is also a double one. For this reason none of the above theories fully cover our case. If one considers the protection due to a coating of the cells, as suggested by Neisser and Freidmann (7), the observed prevention of the electrolyte-arsphenamine reaction is ignored.

The suggestion that prevention is due to adsorptive phenomena between the agglutinating substance and the protective colloid fits our case more nearly, though there are still differences in the manner in which protection results from such an adsorption and the original theories of either Gengou (2) or Henri (4). The former states that such an adsorption between the agglutinating substance and the protective colloid prevents the union with the red cells, while the latter holds that a similar adsorption prevents action of the electrolyte on the agglutinating substance. Considerable debate passed between the proponents of these two possibilities.

However the case may be in the specific instances studied by these two observers, it is evident that in the case of protection from agglutination by arsphenamine, a similar adsorptive (?) process between the arsphenamine and the protective colloid, prevents both its union with the red cells and with the electrolyte. In other words a combination of the two earlier theories fulfills the requirements of our experiments.

As an illustration of the process both of agglutination by arsphenamine and prevention of such action by protective colloids, an analogy with Ehrlich's model of amboceptor may be made. Arsphenamine represents this body, having two "receptors," one for the red cell and another for the electrolyte. The union with either one alone may be demonstrated, on the one hand by the "binding" of arsphenamine by cells in sugar solution, and on the other by the physical changes, precipitation, which result when arsphenamine is diluted with electrolyte solution. When all three components are present, the complex is completed and agglutination results. Protective colloids, however, have a greater affinity for both of the "receptors" of the arsphenamine than the cells or the electrolyte and if present will "plug" them both, preventing, as we have shown in our

experiments, either reaction separately, or the combined reaction and its resulting agglutination.

The wisdom of the use of such gross models may well be debated, but it certainly has the advantage of visualizing the relation between the observed phenomena. If we remember that it is the relation between the phenomena, and not the phenomena themselves that are portrayed, no essential sacrifice of accuracy is made.

CONCLUSION

1. The agglutination of red cells by arsphenamine is inhibited by many hydrophilic colloids.

2. The protective power of certain such substances studied corresponded roughly with their efficiency as expressed by their "gold number."

3. Both phases of the process of agglutination are affected in this inhibition, both the union of the arsphenamine with the red cells and the action of the electrolyte with the arsphenamine.

4. Adsorption phenomena between the protective colloid and the arsphenamine will explain the lack of reaction of the latter with both the other elements, cells and electrolyte, which is necessary for agglutination.

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THE ACTION OF SALICYLATES ON THE UTERUS

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Many textbooks of pharmacology, e.g., Binz (1), Meyer and Gottlieb (2), Sollmann (3) and Cushny (4), mention that abortion sometimes occurs during the treatment of acute rheumatism with salicylates, but that it is uncertain which of these factors is the cause.

Binz gives the results of a series of experiments performed on pregnant rabbits in his laboratory; out of fifteen experiments salicylate caused abortion in five, though the doses were not sufficient to cause any other serious symptoms. Binz endeavors to find some other factor than the salicylates for these effects, such as the temperature of the cages, but is compelled to admit the possibility that salicylate may cause abortion, and advises that it should be given with care in cases where there is any tendency to miscarriage or any history of hemorrhage. We are unaware of any other experimental research on this subject, but Alvarez (6) records stimulation of the movements of the excised intestine with concentrations of salicylate of sodium of 1 in 800, in some cases a primary stimulation being followed by inhibition.

In the following research we have endeavored to investigate the action of salicylate of sodium on the uterus with a view to determining its possible influence in abortion.

PART I. EXPERIMENTAL

a. Action on the excised uterus

Method. The movements of the isolated uterus were recorded by suspension in oxygenated Locke's solution in an apparatus similar to that of Dale and Laidlaw (7). When the movements

207

became regular, or, if they were absent, when the tone became constant, measured amounts of a warm solution of sodium salicylate in Locke's solution were added and the effects recorded.
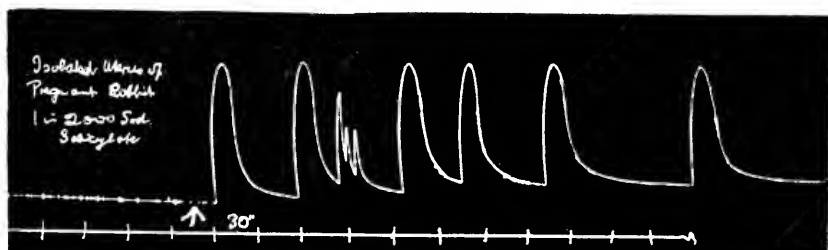
FIG. 1. EFFECT OF 1:2000 SODIUM SALICYLATE ON THE EXCISED UTERUS OF THE PREGNANT RABBIT

It seems that the uterus is slightly more susceptible to the action of drugs at 40°C. than at 37°C., so in most of the experiments the temperature of the Locke's solution was kept at 40°C., which corresponds to the pyrexia of rheumatic fever.
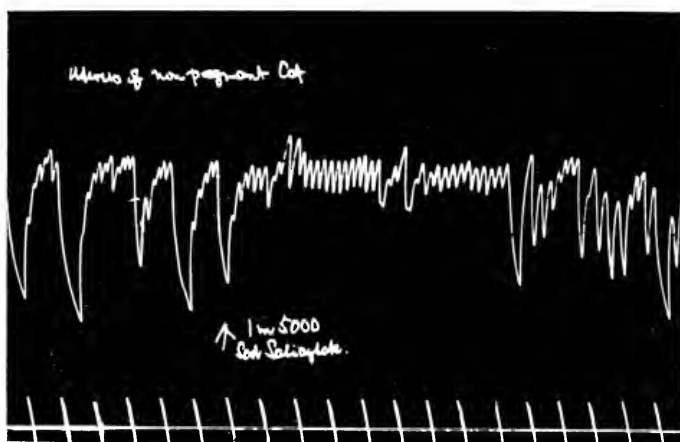
FIG. 2. SLIGHT INCREASE OF TONE OF THE ISOLATED UTERUS AFTER 1:5000 SODIUM SALICYLATE

Results.   Salicylate of soda in sufficient quantity stimulated the uterus, as shown by an increase of its tone or of the frequency or extent of its movements.   The necessary concentration varied

slightly, but 1 in 1000 was always effective. This was as a rule sufficient to induce rhythmical movements in a previously quiescent uterus, but in two experiments it produced a single powerful contraction of short duration but no regular movements. (The latter fact is mentioned as a similar action was seen in some of the experiments on the intact uterus.) In most experiments 1 in 2000 had some stimulant action, and in one (fig. 1). it caused the appearance of rhythmical movements. Lesser strengths had a slight stimulating action or none at all. 1 in 500 sodium salicylate produced primary stimulation, then depression.

All these effects were reversible as the uterus returned to its previous state of movement and tone when the experimental solution was again replaced by Locke's solution.

We obtained the same results on the pregnant and non-pregnant uterus of the cat, rabbit, guinea-pig and rat. The action is therefore presumably directly on the muscle, as it is independent of the predominant action of the inferior mesenteric nerves.

b. Action on the uterus in situ

'*Method.* The movements of the uterus in situ were recorded with Cushny's myocardiograph as described in a previous paper (8). The salicylate of sodium dissolved in warm saline solution was injected into the jugular vein. Twenty-one experiments were performed on the pregnant and non-pregnant rabbit and guinea-pig, the pregnant rat and the non-pregnant cat.

Results. The results of these experiments were not very striking. In two cases a slight but undoubted stimulation was seen after doses of 20 mgm. per kilo., and progressively increasing effects after larger doses. Subsequent experiments showed that any effects with such small doses were quite exceptional, and in the later experiments we gave 50, 100 or even 200 mgm. per kilo. as the initial dose. In more than a third of our experiments salicylates had no apparent effect on the uterus. In the remainder there was an increase in the rate or the amplitude of the movements. The stimulation did not usual'y last for long, in some not much longer than the duration of the injection, in

others five or ten minutes, but rarely longer. It was particularly noticeable that in some animals that had received as much as 300 or 400 mgm. per kilo. in divided doses, the movements of the uterus a few minutes after the last injection were little if any more active than before any salicylate had been given.

In most cases, especially when the effects of salicylates were negative, a subsequent injection of adrenaline, pituitary extract or other uterine stimulant was given. This served to show in
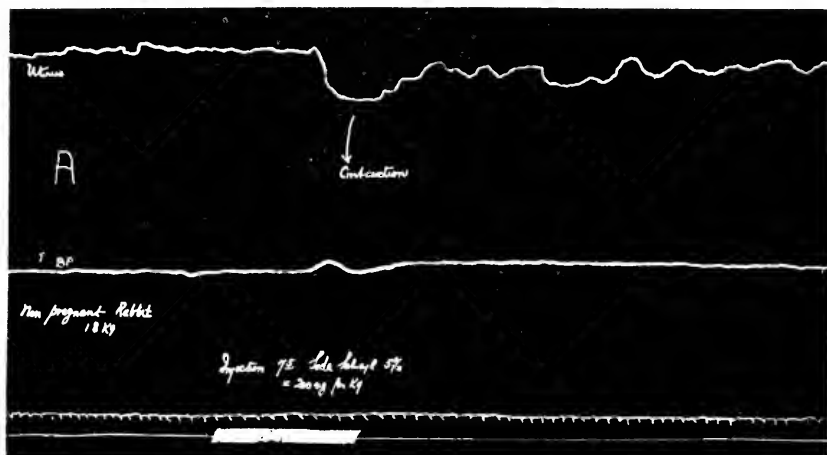
FIG. 3. UPPERMOST LINE SHOWS UTERINE MOVEMENTS, CONTRACTION RECORDED BY DOWNWARD MOVEMENT OF THE UTERUS OF THE NON-PREGNANT RABBIT. EFFECT OF INJECTING 200 MGM. OF SODIUM SALICYLATE PER KILO. LOWEST LINE RECORDS THE INJECTION

the latter cases that the negative results were not due to defects in the method of recording, and in the others by comparison it emphasized the relative inefficiency of the salicylates.

Figure 3 shows the effect of the intravenous injection of 200 mgm. of sodium salicylate per kilo. on the uterus of the non-pregnant rabbit. An increase of movement resulted which had not passed off ten minutes later when an injection was made of 18 mgm. per kilo. of an extract of the skin secretion of the South African clawed toad (Xenopus laevis). It shows (fig.

4) that the contractions produced by the very large dose of salicylate were not maximal.

These large doses of salicylates affect the medullary centres and also slow the heart directly (since the rate is still altered after previous section of the vagi, and it is possible that, apart from a direct action, the uterus may also be affected by changes in its blood supply.
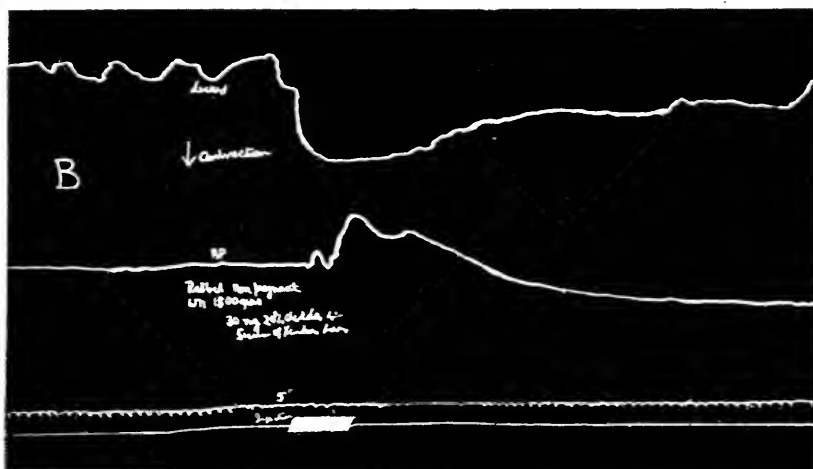
FIG. 4. SAME TRACING AS IN FIGURE 3 AFTER TEN MINUTES. FOR DESCRIPTION SEE TEXT

PART II. DISCUSSION

These experiments show that salicylate of soda has a definite stimulating action on the uterus. This action is not powerful; for instance, on the isolated uterus it is only half as strong as sodium carbonate and possibly less than one fortieth as strong as quinine hydrochloride.

In the treatment of rheumatic fever salicylates are given in large doses, and the percentage in the blood is high. In one case Scott, Thoburn and Hanzlik (9) estimated the concentration in the blood, calculated as salicylic acid, at 0.031 per cent. In terms of sodium salicylate this is about 1 in 2800. Even such a concentration we found to have little action on the isolated

uterus as a rule, although in cases where the uterus was more irritable than usual it did produce some stimulation.

In the experiments on the uterus in situ the maximum effect obtained by us is shown in figure 3. A dose of 200 mgm. per kilo is approximately equivalent to a 200 grain dose for an adult of average weight. It is scarcely likely that even in the most energetic treatment the dose in the blood stream would exceed that given in this experiment. As already stated, it was noticed that when large doses were gradually introduced into the blood, as occurs when salicylates are given therapeutically by mouth, the stimulation of the uterus was much less marked. It is therefore probable that in the treatment of acute rheumatism, unless the uterus is specially sensitive, no greater action will occur than is shown in figure 3. The stimulation did not appear to us to be so pronounced as to suggest that it is likely to be of any great importance in the production of abortion.

It is doubtful what inferences can be drawn from the experiments in which the uterus was more irritable than usual and reacted to smaller doses, for instance, in the intact animal, to 20 mgm. per kilo. This irritability did not seem to be due to mishandling in operative procedure, for considerable quantitative differences were also found in the action of salicylates on other systems. In tracing 3 it will be noticed that 200 mgm. scarcely affect the blood pressure, while in other experiments half that dose produced a decided fall. When salicylates are administered clinically very wide variations are found in the amounts given before toxic symptoms appear, and it is not impossible that there may also be considerable differences in the amounts necessary to influence the uterus.

Whatever be the explanation of the increased irritability of the uterus seen in our experiments, it is well known that this condition occurs in nature, and it is of importance in the causation of abortion. In those women who have the "habit of aborting," it is possible that large doses of salicylates might encourage miscarriage, and our experiments are concerned only with the effects of salicylates on the movements of the uterus, and do not touch on abortion arising less directly; for example,

this treatment not infrequently gives rise to hemorrhage from various organs, and miscarriage may possibly arise in some cases · from bleeding into the uterus.

Abortion may occur during any infectious fever, especially in those in which there is a marked tendency to hyperpyrexia. As far as we can find out from the literature available to us abortion is less common in rheumatic fever than in several other acute fevers, such as pneumonia, scarlet fever and meningitis.

Acute rheumatism is practically universally treated with salicylates, and if these had a greater abortifacient action than our experiments show, the incidence of abortion in rheumatic fever would be greater than it is. In fact since the effect of salicylates is so apparent on rheumatic fever and so slight on the uterus, it is possible that the reason why fewer miscarriages are seen in rheumatic fever than in the other diseases mentioned is because of the rapid control of the former fever by sodium salicylate.

SUMMARY

1. Salicylate of sodium has a stimulating action in the uterus, but usually only in concentrations higher than those found in the blood during the treatment of rheumatic fever.

2. From a consideration of the experiments it is suggested that abortion occurring in rheumatic fever is the result of the fever, and that salicylates have probably little effect unless the uterus is specially sensitive.

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A NOTE ON ADRENALIN HYPERGLYCEMIA IN MAN

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Although the glycogenetic action of adrenalin in animals and man is well established, the mechanism by which adrenalin mobilizes glucose in the circulation is as yet undetermined. Tatum (1) has recently studied the relation of acidosis or hepatic asphyxia to adrenalin hyperglycemia, and has reviewed the literature on this subject. He sums up his impressions as follows:

We are forced to conclude that epinephrine glycogenolysis cannot be satisfactorily explained on the basis of hepatic asphyxia or acidosis. The real mechanism of epinephrine mobilization of carbohydrates therefore is as yet undetermined.

Previously, Lamson (2) studying in dogs the acute polycythemias produced by intravenous injections of adrenalin, showed that the increase in the number of erythrocytes per cubic millimeter of blood following the adrenalin is brought about by the mechanism of a concentration in the blood due to loss of fluid into the liver. Lamson describes this mechanism as follows:

An obstruction to the venous outflow of the liver; an increase in portal pressure; passage of (blood) fluids into the liver lymphatics, causing a swelling of the liver and concentration of the blood, followed by a gradual return of the fluid *via* the thoracic duct to the general circulation, with a return of the red count to normal.

It occurred to us that this mechanism of blood concentration following intravenous adrenalin, as observed by Lamson in dogs, might be at least one factor in the mechanism of adrenalin hyperglycemia. Consequently, the present clinical study was under-

taken to answer two points: first, is the concentration of the blood observed in laboratory animals following adrenalin paralleled in man; and secondly, is such a blood concentration a factor in the mechanism of adrenalin hyperglycemia?

Although neither of these points was answered in the affirmative, the results appear of sufficient interest to justify a brief report. Since the maximum dose of adrenalin which could be tolerated in man was found to be infinitely smaller than the physiological dosage for dogs, concentrations of the blood in human subjects comparable to those observed in dogs following adrenalin were not produced. The hyperglycemia produced by adrenalin was found to be independent of blood concentration changes. The maximum intravenous dosage of adrenalin in man, the systemic effects of such dosage, and the wide divergence between the dosage of adrenalin tolerated in the experimental animals and in man, are touched upon in our results. The influence of the intravenous adrenalin on the concentration of the nitrogenous metabolites, creatinine and blood urea nitrogen, was also observed.

METHOD

Following Lamson (2) the percentile difference in the erythrocyte count before and after the adrenalin served as an index of blood concentration, due to loss of fluid from the blood plasma.

Clinical cases were selected from the medical wards, post alcoholics, hemiplegiacs or convalescents—cases free from anemia and endocrine disturbances. Following rest in bed overnight, without food, 15 to 20 cc. of blood was collected by venepuncture in oxalate tubes. An erythrocyte count was obtained at the same puncture. The adrenalin was then administered. Similar samples of blood for chemical analysis and red counts were taken precisely fifteen minutes (or in some cases thirty minutes) after the adrenalin. The red counts were all done by the same man, and the average of three separate counts used on each sample. The blood analyses were done as soon as possible. The blood sugar was estimated by the method of Lewis and Benedict (3), the creatinine by the method of Folin (4) and the blood urea

nitrogen by Van Slyke and Cullen's modification of Marshall's urease method (5).

DOSAGE AND REACTION

The first four cases were given 0.25 cc. of 1:1000 solution of adrenalin chloride (Parke, Davis & Company), subcutaneously in the deltoid region. No unpleasant reactions occurred. The next seven cases were given 0.25 cc. of the same solution intravenously, usually in 5 cc. of warmed normal saline. The twelfth case received 0.33 cc. of 1:1000 adrenalin chloride in 5 cc. warmed normal saline, intravenously.

Immediately upon the injection of the drug into the veins, the patients exhibited deep, sighing respiration, loss of color, small, rapid, and irregular pulse, with headache and a feeling of anxiety, lasting for some time. The reactions averaged from three to five minutes, and though quite severe, even alarming, left no sequelae, and passed off completely within ten minutes.

In the last case, however, the reaction was so severe as to make further experimentation unjustifiable. The patient was a robust young man, apparently completely recovered from a very mild case of illuminating-gas poisoning. He received 0.33 cc. of 1:1000 adrenalin chloride, in 5 cc. warmed normal saline, intravenously. A very severe reaction occurred before the needle was withdrawn. The face assumed a waxen, death-like pallor, the radial pulse became impalpable for several minutes, and respiration was completely suspended for over a minute, after which there were several violent, sneeze-like expiratory movements. Although the severity of the reaction passed off within five minutes, the observers agreed that his condition appeared such that it seemed not unlikely the patient might die. Consequently, further intravenous injection of adrenalin was abandoned. Our experience leads us to feel that 0.33 cc. of 1:1000 adrenalin chloride, diluted in 5 cc. of warmed normal saline, is beyond the physiological intravenous dosage of the drug and probably the maximum dosage that can be tolerated in safety by human subjects.

RESULTS

The table appended contains all the data obtained.   It is regrettable that the reactions necessarily limited the cases to too small a number for arriving at any very definite conclusions.

Effect of adrenalin on the concentration of red blood cells and metabolites

NUMBER	AMOUNT	ADMINISTERED	PER CENT RED CELLS			PER CENT SUGAR			PER CENT CREATININE			PER CENT B. U. N.		
			Fasting	15 minutes	60 minutes	Fasting	15 minutes	60 minutes	Fasting	15 minutes	60 minutes	Fasting	15 minutes	60 minutes
	cc.													
1	0.25	Subcut.	100	117.4	91.3	100	110.1	128.3	100	91.5	93.5	100	106.1	96.5
2	0.25	Subcut.	100	102.0	102.0	100	103.4	106.4	100	69.5	94.9	100	108.7	108.7
3	0.25	Subcut.	100	96.4	103.6	100		105.0	100		100.0	100	84.8	93.5
4	0.25	Intraven.	100	90.9	103.3	100	120.5	136.6	100	108.3	117.0	100	134.2	154.6
5	0.25	Intraven.	100	91.7	83.9	100	138.0	116.0	100	123.0	100.0	100	142.5	104.8
6	0.25	Intraven.	100	108.9	103.5	100	104.4	85.1	100	100.0	100.0	100	112.7	107.0
7	0.25	Intraven.	100	97.2	88.7	100	119.5	96.2	100	94.7	84.0	100		76.3
8	0.25	Intraven.	100	93.1	79.1	100	106.6	105.0	100	96.1	80.8	100	79.1	79.1
9	0.25	Intraven.	100	91.8	89.8	100	118.5	117.4	100	92.6	92.8	100		89.6
				30 min.			30 min.			30 min.			30 min.	
10	0.25	Intraven.	100	117.0	97.6	100	99.1	95.5	100	80.5	49.5	100	126.2	105.8
11	0.25	Intraven.	100	103.1	114.9	100	106.0	98.0	100	66.3	72.0	100	97.7	90.7
12	0.33	Intraven.	100	118.5	97.3	100	111.7	103.2	100	105.9	100.0	100	117.8	107.6

From the table, however, several points may be noted.   In the first place, it is apparent that the red cell concentration exhibits no consistent tendency to duplicate the results which Lamson observed in dogs.   The twelve cases are evenly divided between six which show an increased, and six a decreased concentration of the blood, as indicated by the red counts.   No constant loss of plasma is produced.   The answer to our first question, then, is in the negative; *the adrenalin polycythemia brought about by concentration of the blood through loss of fluid into the liver as observed in dogs, cannot be duplicated in man.* Of the three metabolites, the blood sugar alone showed a consistent reaction.   In all but one case, in which it remained practically unchanged, the blood sugar increased, following the

adrenalin, relative hyperglycemia being present at fifteen minutes, and in most cases an hour after the adrenalin injection. The relatively small increment in the blood sugar (3.4 to 38 per cent) following adrenalin in man, as compared to laboratory animals, may be due to the smaller dosage tolerated in human subjects. No tendency for the changes in blood sugar to parallel the changes in blood concentration was noted.

Both the blood creatinine and the blood urea nitrogen are seen to show very wide and inconsistent fluctuations both above and below their original level. We are unable to suggest any interpretation of these findings.

<center>DISCUSSION</center>

There are several points of interest suggested by these results. In the first place, the severe systemic reaction produced by 0.33 cc. of 1:1000 solution of adrenalin chloride, intravenously, indicates that amount as the maximum dosage for human subjects. This corresponds to approximately $\frac{1}{150}$ of the physiological dosage per kilo in dogs,[1] and illustrates the marked variation in sensitivity to the drug, comparing man to laboratory animals, and the fallacy of drawing conclusions concerning the action of the drug in man from its effect on laboratory animals.

That blood concentration is not a factor in the mechanism of afdrenalin hyperglycemia is further substantiated by a consideration of the distribution of the sugar of the blood between the corpuscles and the plasma. The following analyses of human blood are cited from McLeod (7):

Distribution of blood sugar between corpuscles and plasma

PER CENT IN WHOLE BLOOD	PER CENT IN PLASMA	PER CENT IN CORPUSCLES	AUTHOR
0.12	0.18	0.121	Bailey (8)
0.135	0.135	0.135 ⎫	Gradwohl and Blavais (9)
0.102	0.099	0.102 ⎬	
0.098	0.105	0.082	Tachau (10)
0.094	0.098	0.089	Rona and Dahlin (11)

[1] Dogs are more tolerant to adrenalin when under ether.

It is evident from the above that in human blood there is a very slight difference in the concentration of sugar in the cells and the plasma. This being the case, the loss of even a very great percentage of the plasma from the circulation would leave approximately the same amount of sugar per unit volume of blood. Consequently, even if the adrenalin has been found to produce an appreciable change in blood concentration, it would *a priori* be impossible to attribute the adrenalin hyperglycemia to this mechanism.

CONCLUSIONS

1. A concentration of the blood following adrenalin as observed in dogs is not paralleled in man, the maximum intravenous dosage being employed.

2. The mechanism of adrenalin hyperglycemia in man cannot be explained on the basis of a change in blood concentration due to the adrenalin, but occurs independently of such a change.

3. The maximum intravenous dosage of adrenalin in man is approximately 0.33 cc. of a 1:1000 solution in a 70 kilo man, which corresponds to about $\frac{1}{180}$ of the physiologic intravenous dose per kilo in dogs.

4. Intravenous adrenalin caused a wide and inconsistent fluctuation in the concentration of blood creatinine and blood urea nitrogen.

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THE DETERMINATION OF THE CIRCULATION TIME IN RABBITS AND DOGS AND ITS RELATION TO THE REACTION TIME OF THE RESPIRATION TO SODIUM CYANIDE

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Our interest in this problem arose from the fact that in the course of various investigations we have injected sodium cyanide intravenously and always obtained a stimulation of the respiration within a few seconds when an appropriate dose was used. The rapidity of the response led us to believe that the reaction time to sodium cyanide was occupied principally in the transport of the cyanide from the site of the injection to the respiratory center. In other words, the time required for the drug to diffuse out of the blood and into the cells of the center, together with the alteration in the character of the chemical processes in the cells of the center, which result in stimulation, all seemed to occupy an exceedingly small fraction of the reaction time. If this proved to be true, it occurred to us that the reaction time of the respiratory center to sodium cyanide might be used in determining the circulation time. Such a method would present obvious advantages over any previous method which has been proposed for determining the circulation time because it could be done on the intact animal without any operative procedure and without any method of chemical or electrical testing of the blood. In fact, the procedure would require simply a hypodermic syringe and a method of determining the exact time of stimulation of the respiration. Furthermore, since the method does not involve bleeding or any injury whatever to the animal, it could be used over and over again in the same animal and might also be applicable clinically to the determination of the

221

circulation time in man. In order to determine the relation of the reaction time to the circulation time in our animals, we employed various methods that have been suggested from time to time by different investigators[1] in the determination of the circulation time. We have found all of these methods to be somewhat unsatisfactory and inaccurate and we have devised a new technique for the determination of the circulation time in animals.

EXPERIMENTAL METHODS

In the work with rabbits, no anesthetic was used since no operative procedure was involved. In dogs the operative part was done under local anesthesia. The method which we have used in determining the circulation time differs from previous methods in the nature of the substances used for testing and in the method of bleeding.

Substances used for testing

We have employed lithium chloride, lithium benzoate, lithium acetate and hexamethylene tetramine and have compared the results obtained with these substances with those obtained when sodium ferrocyanide is used. The reasons for employing these substances were:

1. The ease of testing for these substances in the blood and the delicacy of the tests.

2. The absence of marked pharmacological effects following their administration.

From the standpoint of ease of testing, the lithium salts are decidedly the most attractive. In testing for lithium in the blood, all that is necessary is to obtain a fraction of a drop of blood which is then treated with a drop of concentrated hydrochloric acid and inspected spectroscopically. The amount of lithium contained in the platinum loop of material, which will

[1] We cannot distinguish between the action of hydrocyanic acid and sodium cyanide when given intravenously because when sodium cyanide is administered it is immediately decomposed into hydrocyanic acid and sodium carbonate by the carbon dioxide of the blood.

give the characteristic red line when examined with the spectroscope, is less than 0.000,002 mg. Lithium chloride, in strong solution, injured the vein at the site of injection, and therefore the organic salts, namely, the benzoate and acetate, are preferable to it. The amount of lithium injected in the rabbit was 0.18 cc. of a 50 per cent solution per kilo. in an average of 1.08 seconds, and in the dog was 0.6 cc. of a 24 per cent solution per kilo. in an average of 1.28 seconds. All of the lithium salts possess the disadvantage that when injected intravenously in the dosage used, cause dogs to vomit. The vomiting, however, occurs after all observations on the circulation time are completed.

Hexamethylene tetramine can also be readily tested for in the blood in extremely small amounts. The method used in making this test is to dilute the blood with a small amount of water and acidify with diluted sulphuric acid and distill, using a small distillation apparatus. The distillate is tested for formaldehyde, using the Hehner test which consists of treating 1 cc. of the distillate with 1 cc. of concentrated sulphuric acid, the latter agent containing a very small amount of ferric chloride, and adding to this mixture a small amount of caseinogen in solid form. A pink to purple color develops within a few minutes, depending on the concentration of the formaldehyde in the distillate. The test is so delicate that it is necessary to run a blank test on the apparatus before each actual test is made, in order to be sure that the apparatus is thoroughly free of formaldehyde and hexamethylene tetramine. The test is sensitive to approximately one part in fifty million of formaldehyde in the distillate. If the formaldehyde is of a greater concentration than one in ten thousand, a negative test is also obtained but this never occurs in work on the circulation time. The hexamethylene tetramine in the rabbit was used in a dosage of 0.38 cc. of a 40 per cent solution per kilo. injected in an average of 1.6 seconds, and in the dog 0.6 cc. of a 33 per cent solution per kilo. injected in an average of 1.25 seconds. This dosage of the drug produced no discernible pharmacological effects whatever which together with the delicacy of the test, makes the

substance perhaps the most favorable for determining the circulation time in animals by chemical means.

The method of bleeding

The method of bleeding and of injecting varied in rabbits and dogs. In the rabbit, the injection was made into one marginal ear vein and the bleeding was from the marginal vein of the opposite ear. In the dog, a cannula was placed into each external jugular vein, under local anesthesia, the injection was made into the left external jugular vein, and the bleeding was from the right external jugular vein. Both in the case of rabbits and dogs, a modification of the Hürthle kymograph was used for collecting the samples of blood and to record the respiration and procedures. The Hürthle kymograph was arranged so that the paper was in a horizontal position. A strip of rubber adhesive was pinned to the kymograph paper at the margin. On this rubber adhesive were placed receptacles for receiving the blood. In the case of rabbits, these receptacles consisted of small watch crystals, approximately 18 mm. in diameter. These were placed on the rubber adhesive with an interval of about 1 mm. between the receptacles. In the case of dogs, the receptacles consisted of homeopathic vials, approximately 18 mm. in diameter, the necks of which were cut off so that the remaining tube was approximately 25 mm. in height. The kymograph was run at a speed of approximately 18 mm. per $\frac{1}{5}$ second, so that each of these receptacles represented the bleeding for only $\frac{1}{5}$ second. The time record was taken with a Jacquet chron graph, which recorded $\frac{1}{5}$ second. The syringe used for the injection was so equipped that pressure of the thumb on the piston on starting the injection completed a circuit which actuated a magnet connected with a writing point. The beginning and duration of injection was therefore automatically recorded and the injections were made as rapidly as possible. The time was always figured from the beginning of the injection.

In case the sodium cyanide reaction time was to be determined in addition to the circulation time, arrangement was made to

record the respiratory movements on the same record. This was done by means of a volley ball bladder placed on the chest and enclosed in a piece of muslin, and the respiratory movements recorded by means of a tambour by air transmission. Several determinations of reaction time to sodium cyanide were made just before and several immediately after the determination of circulation time, and in many cases the reaction time was also determined simultaneously with the circulation time.
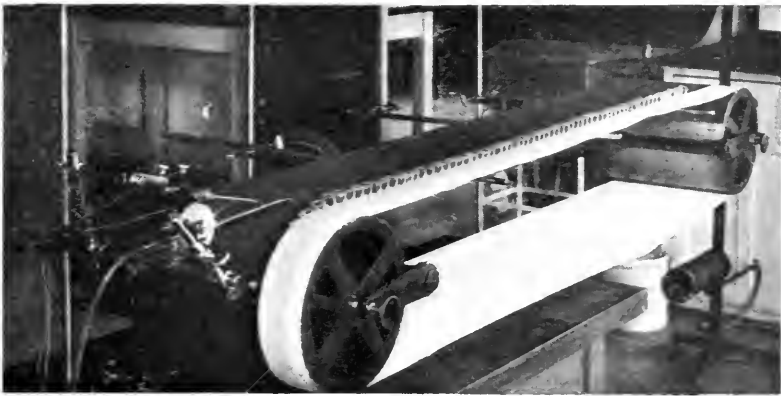
Fig. 1

Just before the injection is started, the blood must be flowing very freely from the vein from which the blood is taken. There must be no obstruction to the outflow. In many cases, the solution injected contained the lithium salt, hexamethylene tetramine, and sodium cyanide in order to get a direct comparison on the same animal, using both of the first mentioned substances, and to determine simultaneously the relation of the circulation time to the reaction time of the respiratory center to the cyanide. The entire procedure lasted about 15 to 20 seconds.

In order to give a concrete idea of the work, we will present the detailed protocolls of two experiments, one on the rabbit and one on the dog.

Rabbit; experiment 16; male; 2.3 kilos

PRO-CEDURE	TIME	INJECTION	INJEC-TION TIME	RE-ACTION TIME TO NaCN	CIRCU-LATION TIME
1	2:43	0.3 cc. N /50 NaCN	0.4	3.6	
2	2:50	0.3 cc. N /50 NaCN	1.2	4.3	
3	3:00	0.3 cc. N /50 NaCN	0.9	4.0	
4	3:05	1.0 cc. 20 per cent Na$_4$Fe (CN)$_6$	1.3		4.0
5	3:08	1.0 cc. 40 per cent (CH$_2$)$_6$N$_4$	1.2		3.8
6	3:15	0.5 cc. LiCl 50 per cent	0.7		4.8
7	3:20	0.3 cc. N /50 NaCN	0.6	4.7	
8	3:24	0.3 cc. N /50 NaCN	1.2	4.8	

Dog; experiment 10; female; 8 kilos

	TIME	PROCEDURE	INJECTION TIME	REACTION TIME TO NaCN	CIRCULATION TIME	HEART RATE PER MINUTE	
						Before injection	After injection
	2:15–2:20	Tied dog down and cannulas inserted under local anesthesia					
1	2:20	Injected 0.75 cc. N /50 NaCN	0.8	7.5		132	111
2	2:30	Injected 0.75 cc. N /50 NaCN	0.5	6.0		126	120
3	2:40	Injected 0.75 cc. N /50 NaCN	0.6	5.8		116	102
4	2:52	Injected 0.75 cc. N /50 NaCN	2.0	6.6		132	128
5	3:05	Injected 5.0 cc. {0.75 cc. N /50 NaCN / LiBenzoate 23 per cent / (CH$_2$)$_6$N$_4$ 33 per cent}	1.4	5.8	Li 7.0 H 7.0	124	132
6	3:15	Injected 0.75 cc. N /50 NaCN	0.6	5.7		136	99
8	3:28	Injected 0.75 cc. N /50 NaCN	0.6	6.3			
9	3:29	Vomited					

RESULTS

The results can be most briefly presented in the form of the following tables. The time in every case is given in seconds. The tables show the reaction time of the respiratory center to sodium cyanide in rabbits and dogs immediately before the determination of the circulation time. Then the circulation time

was determined, as indicated in the tables, using the three substances. In the rabbit, the sodium ferrocyanide, hexamethylene tetramine and lithium salt were each injected separately, and following the bleeding the reaction time to sodium cyanide was again taken. In the dog, the lithium salt, hexamethylene tetramine and sodium cyanide were all given simultaneously in a single injection. We should like to point out again that none of the animals were under general anesthesia. In the case of rabbits, no anesthetic was used and in the case of dogs we employed only local anesthesia during the operative part.

<div align="center">DISCUSSION OF RESULTS</div>

<div align="center">*The circulation time*</div>

The circulation time from ear vein to ear vein in the rabbit (table 1) for all three substances used gives an average of 4.71 seconds. The average circulation time from jugular to jugular in the dog (table 2) for lithium salt and hexamethylene tetramine is 7.8 seconds. It will be noted on examining the tables that the circulation time for a given animal for different substances does not vary greatly, the maximum variation being 2.1 seconds in the case of the rabbit (experiment 10). In the case of the dog, the maximum variation is 1 second (experiment 5). In most cases there is remarkably close agreement in the results with a given animal. On comparing the variation in different animals, we have used the averages obtained for each animal and here we find the maximum variation in the rabbit to be from 3.6 to 7.2 seconds or 100 per cent. In only three experiments did the circulation time in the rabbit exceed 5 seconds. In the case of the dog, the agreement between the circulation time, as determined with lithium salt and hexamethylene tetramine, shows remarkable agreement. In most cases, the first bottle, representing the bleeding of $\frac{1}{5}$ second, which gave a positive test for lithium salt, also gave the first positive test for hexamethylene tetramine. The results are very striking from this standpoint. The maximum variation in the circulation time in the dog was from 4.9 to 10.2 seconds (108 per cent).

The circulation time, as determined by us, is very much faster than that found by Vierordt whose figures for the circulation

TABLE I
Rabbits

EXPERIMENT NUMBER	WEIGHT	SEX	REACTION TIME TO NaCN (BEFORE BLEEDING)				CIRCULATION TIME				NaCN REACTION TIME (AFTER BLEEDING)		
			1	2	3	Average	Na₄Fe(CN)₆	(CH₂)₆N₄	LiCl	Average	1	2	Average
	kilos												
3	1.38	Male		4.3	5.2	4.7					5.0	4.2	4.6
4	1.96	Male	4.2	4.6		4.4	4.4		4.5	4.45	3.4	3.4	3.4
5	2.26	Male	4.0	4.2		4.1			5.8	5.8	3.2		3.2
6	2.08	Male	5.0			5.0							
7	2.1	Female	4.7	4.0		4.35		9.0*			3.7		3.7
8	2.1	Male		5.0	4.4	4.7							
9	1.7	Female	3.4			3.4		3.6		3.6	3.2	4.2	3.7
10	2.4	Female	4.5	3.4	3.4	3.77	5.6		3.5	4.55	4.2		4.2
11	1.92	Female	3.8	3.0		3.4	3.8	3.4		3.6	2.8	2.0	2.4
12	1.54	Female	3.3	3.4		3.35	4.4			4.4	5.0		5.0
13	2.0	Female	3.6	3.5		3.55			3.7	3.7	3.4		3.4
14	1.83	Female	3.4	3.2		3.3	4.6		4.7	4.65	3.2		3.2
15	1.7	Male	4.4	4.8	5.0	4.73							
16	2.3	Male	3.6	4.3	4.0	3.93	4.0	3.8	4.8	4.2	4.7	4.8	4.75
17	1.9	Female	3.5	3.5		3.5							
18	2.3	Female	3.8	4.2	3.8	3.93	5.0	4.5		4.75	4.8	5.0	4.9
19	2.64	Male	3.8	3.6		3.7	3.8			3.8	4.0	6.1†	5.1
20	2.6	Male	3.6	3.8	4.0	3.8							
21	2.0	Male	4.0	4.8	4.7	4.5							
22	2.4	Male	3.6	3.4	3.4	3.47							
23	2.55	Male	3.6	3.9		3.75							
25	2.46	Male						7.2		7.2			
26	2.46	Male						4.2		4.2			
27	2.0	Male						7.0		7.0			
Average..	2.11					3.97	4.45	4.81	4.5	4.71			3.96

* Poor bleeding: not counted in calculating the average.
† Convulsion.

time are the ones usually quoted by textbooks on physiology. Vierordt's results were as follows: Horse 28.8, dog 16.32, goat 14.14, rabbit 7.46.

TABLE 2

Dogs

EXPERIMENT NUMBER	WEIGHT	SEX	REACTION TIME TO SODIUM CYANIDE (BEFORE BLEEDING)						REACTION TIME DURING BLEEDING	CIRCULATION TIME			REACTION TIME TO NaCN (AFTER BLEEDING)			
	kilos		1	2	3	4	5	Average		Li salt	$(CH_2)_6N_4$	Average	1	2	3	Average
1	8.0	Male	8.9	7.2	8.1	8.0	8.4	8.12	.							
2*	8.0	Male	7.8	8.1	5.8	8.2	8.7	7.72								
3	7.5	Male	9.2	8.3	9.6	8.4	8.3	8.76								
4	8.5	Male	9.6	9.8			9.4	9.6								
5	9.0	Female	9.5	9.6	10.1			9.73								
6	10.0	Female	7.7	7.9	9.0	9.7		8.57		8.4	9.4	8.9	8.0	8.2		8.1
7	7.5	Female	8.4	8.4	8.5			8.43	8.0	7.5	7.5	7.5	9.3			9.3
8	11.5	Male	9.3	9.3	9.3			9.3	9.5	6.6		6.6	10.3	10.5	10.4	10.4
9	8.5	Male	13.8	13.5	11.4	12.8	13.5	13.0	10.5	9.5	9.0	9.25	11.6	13.6		12.6
10	8.0	Female	7.5	6.0	5.8	6.6		6.47	5.8	10.2	10.2	10.2	5.7		6.3	6.0
11	4.5	Female	6.8	7.1	6.9			6.93	7.5	7.0	7.0	7.0	7.6	7.6		7.6
12	8.5	Female	13.6	13.8	11.5	12.5		12.8	9.7	9.5	9.0	9.25	7.7	7.0	7.8	7.5
13	10.0	Female	13.4	10.2	10.4	10.5		11.12		6.6	6.6	6.6				
14	5.6	Male	6.6	6.3	6.5			6.46					9.0			9.0
15	9.5	Male	6.4	6.4	6.7			6.5					6.8	6.2		6.5
16	7.0	Male	7.8		8.0			7.9	6.0				7.0	7.6		7.3
17	7.6	Female	6.2		5.4			5.8		4.9	4.9	4.9	7.4	7.9		7.65
Average	8.2							8.66	8.14	7.8	7.95	7.8				8.36

* Experiments 1 and 2 were done on the same dog on different days.

Stewart (1) determined the circulation time in rabbits from jugular to jugular and found it to vary from 4.1 to 6.1 seconds— the average of five determinations being 5.1 seconds. Our average of 4.71 is somewhat shorter, especially when we consider that our figure is the circulation time from ear vein to ear vein. Moreover, Stewart's animals received chloral urethane or curare. Stewart states that he was not particularly interested in the absolute circulation time but in the relative circulation time in different organs. Wolff found the average circulation time in rabbits to be 5.5 seconds from jugular to jugular vein. He used sodium ferrocyanide and bled on to sized paper.

In the dog the circulation time, which we have found, is very much faster than that found by any previous investigator. We find the average circulation time from jugular to jugular in dogs to be 7.8 seconds in animals varying from 4.5 to 11.5 kilos. As far as our observations go, there is no relation between body weight and circulation time either in the rabbit or dog. Vierordt places the jugular to jugular circulation time in dogs at 16.32 and this figure has been generally accepted. According to our work, the circulation time is less than half that found by Vierordt. We explain the difference as due to more accurate methods of recording the injections and of bleeding, and especially to the nature of the substances which we have employed. The tests for lithium salts and hexamethylene tetramine in the blood are far more delicate and definite in minute amounts than any substances previously used. It is obvious that the material injected into a vein will become diluted with blood as the vein discharges into a larger vein, in all the chambers of the heart and in the lungs, and the first jot of blood to reach the bleeding point will not contain more than very minute amounts of the substance. After a lapse of a few seconds, the concentration of the substance injected in the blood at the bleeding point rises rapidly to a maximum and then probably decreases again as the material becomes mixed with the whole volume of the blood. For this reason the circulation time will be found the shortest when a substance is used which gives a perfectly definite test in the lowest concentration. The same reasoning applies in the case

of sodium chloride as used in the electric method of Stewart. Here also the salt will have to build up a concentration at the "bleeding point" sufficient to affect the readings.

As to the relation of pulse rate to circulation time, Vierordt calculates that there are approximately 27 heart beats during a complete circulation time. Our results show that this is not the case and we agree with subsequent investigators that there is no constant relation between heart rate and circulation time. In three experiments, in which the heart rate was observed, the number of heart beats during the period of the circulation time varied from 9.25 to 16.4.

Reaction time of the respiration to sodium cyanide

It is of general interest to know how quickly a substance is distributed over the body on intravenous injection and how quickly a substance may pass from the blood into the tissues in sufficient quantity to so alter the chemical processes in the cell as to effect a change in functional activity. It is well known that the diffusibility of different substances through membranes both living and dead varies greatly. Membranes present extremely little resistance to the passage of carbon dioxide, oxygen, urea, etc., whereas other substances pass through with great difficulty, probably due to their chemical as well as their physical nature.

The rapidity of action of the cyanides has been known since the first observations on their toxicological action. In fact, their popular reputation as the deadliest of poison is based on their rapidity of action and not on the smallness of the lethal dose.

In the course of previous investigations in this laboratory, we have been greatly impressed with the constancy and rapidity of stimulation of the respiration following the intravenous injection of sodium cyanide. When used in proper dosage, the effect passes off within less than one minute as a rule and the injection can be repeated any number of times, each injection being followed by stimulation. No deleterious effect on the animals is noted as a result of its repeated administration.

Gasser and Loevenhart (3) studied the rapidity of response of the medullary centers to the injection of sodium cyanide from the standpoint of determining the mechanism of response of these centers to reduced oxidation. They concluded from their work that reduced oxidation per se stimulates the centers and that the stimulation could not be due indirectly to accumulation of acid products of incomplete oxidation. They were only interested, however, in obtaining approximate results to prove this point and the reaction time given by Gasser and Loevenhart is slow in the light of our more accurate results given in tables 1 and 2. We find that the average reaction time of the respiration to sodium cyanide in twenty-one rabbits (47 injections) is 3.97 seconds, the cyanide being injected into the marginal ear vein. The shortest reaction time in our experiments is 3.3 seconds and the longest 5.2 seconds. The average reaction time to sodium cyanide administered by the jugular vein in seventeen dogs (61 injections) is 8.66 seconds. The shortest reaction time is 5.4 and the longest is 13.8 seconds. It will be seen from tables 1 and 2 that the weight of the individual bears no relation to reaction time either in the rabbit or dog.

Experiments 20 and 21 were performed to determine the variation in the reaction time to sodium cyanide in rabbits over a period of two hours in the same individual. The dosage was 0.3 cc. 0.02 N sodium cyanide.

TIME OF INJECTION	REACTION TIME	
	Experiment 20	Experiment 21
m nutes	seconds	seconds
0	3.6	4.0
10	3.8	4.8
20	3.3	4.8
50	3.4	4.7
110	4.0	4.6
Average..............	3.62	4.58

The greatest variation was 0.8 of a second.

Experiment 22 was performed to study the variation in the reaction time from day to day in the same rabbit. The dosage

was 0.3 cc. of 0.02 N sodium cyanide. The results were as follows:

DAY	TIME	REACTION TIME
		seconds
Monday..	2:00	3.6
Tuesday.......................................	3:00	3.4
Wednesday.....................................	3:00	3.4
Thursday......................................	2:15	3.4
Friday..	2:20	3.9
Average.......................................		3.54

The greatest variation was 0.5 of a second. Tables 1 and 2 gave a large number of data bearing upon the variation in reaction time of individual animals to repeated injections. The variations in the reaction time suggest that the phase of the respiration at the time of injection may play a rôle in the reaction time. We have no experimental data on this point. The reaction time of the respiration to sodium cyanide is therefore fairly constant.

Duration of injection

Obviously, the duration of injection of the cyanide has a marked effect on the reaction time. It was clearly brought out in many experiments that slow injections delay the reaction time markedly and accurate observations can only be made when the injection requires one second or less. The more rapid the injection, the more satisfactory will be the result.

Dosage

Above a certain optimum, the reaction time is independent of the dosage. This optimum is approximately 0.75 cc. 0.02 N sodium cyanide in dogs. In experiment 16, the injection of 0.75 cc. 0.02 N cyanide in 0.8 second gave a reaction time of 7.8 seconds whereas 0.5 cc. of 0.02 N injected in 1.2 seconds gave 21.6 seconds, 1.0 cc. in 0.7 second gave 8.0 seconds.

In experiment 17, 0.75 cc. 0.02 N gave a reaction time of 6.2 seconds and 0.5 cc. gave 7.8 seconds. Our average dose of the

cyanide in rabbits was 0.17 cc. 0.02 N cyanide per kilo and in dogs 0.082 cc. 0.02 N cyanide per kilo.

The site of injection

Four experiments were performed to determine the effect of the site of injection on the reaction time in dogs. In experiments 6 and 10, the reaction time on injection into the external jugular vein and carotid artery were compared:

EXPERIMENT NUMBER	EXTERNAL JUGULAR VEIN	CAROTID ARTERY	CALCULATED CIRCULATION TIME FROM EXTERNAL JUGULAR VEIN TO CAROTID
6	8.57	1.5	7.07
10	6.47	2.7	3.77

In the same manner, the circulation time through any organ can be determined by difference using the cyanide method. One experiment was performed to determine the difference in the reaction time in the same animal when injection was made into the external jugular vein and into the saphenous vein:

EXTERNAL JUGULAR VEIN	SAPHENOUS VEIN
8.38	9.52

As was to have been expected, the reaction time was appreciably longer when the injection was made into the saphenous vein.

Relation of pulse rate to reaction time

We find no relation between the pulse rate and reaction time and this is also true of circulation time, as has been pointed out. The cyanide injections usually slow the pulse rate in unanesthetized dogs in the dosage here used. Thus, in twenty-five cases the cyanide slowed the pulse in twenty instances, increased the pulse in two and had no effect in three cases. The average increase was 3.5 beats per minute, the largest being five beats per minute. The average slowing was thirty per minute and

the greatest decrease in the pulse rate was eighty-five. In the latter case, the original pulse rate was one hundred thirty-five and decreased to fifty following the injection. This decrease in the pulse rate is very transitory and the pulse regains the normal rate within thirty seconds.

The effect of bleeding on the reaction time

In all the work on the circulation time, the reaction time was taken several times before the determination of the circulation time and in most cases several times thereafter. We, therefore, had opportunity in a large number of animals to determine the effect of bleeding although the amount of haemorrhage could not be accurately determined in these experiments. The amount of bleeding in the rabbit varied greatly in the work on the circulation time. We, however, estimate that the average bleeding in rabbit was approximately 10 cc. of blood. The average weight of our rabbits was 2.1 kilos and the bleeding was approximately 0.5 per cent of the body weight. In dogs the bleeding was approximately 1 per cent of the body weight.

The average reaction time before and after bleeding was as follows:

	REACTION TIME	
	Rabbit	Dog
	seconds	*seconds*
Before bleeding..................................	3.97	8.66
After bleeding..................................	3.96	8.36

The effect of bleeding 0.5 per cent of the body weight in rabbits and 1 per cent of the body weight in dogs is therefore without effect on the reaction time.

Ether anesthesia and reaction time

The dosage of 0.02 N sodium cyanide required to stimulate the respiration before and during ether anesthesia in the surgical stage and the reaction time in each case is shown in the following table:

EXPERIMENT	BEFORE		DURING	
	Required	Reaction time	Required	Reaction time
	cc,		cc.	
2	0.5	7.72	0.75	4.87
3	0.5	8.76	1.36	6.19
4	0.58	9.6	1.86*	
8	1.0	9.3	2.50*	
			3.0	10.4
" 9	0.74	13.0	2.0*	
10	0.75	6.47	1.5	6.7

* In these instances stimulation of the respiration did not occur.

In all cases the dosage of sodium cyanide had to be increased in order to effect stimulation of the respiration. The increase required varied from 50 to 300 per cent. In experiments 2 and 3 there was a decrease in the reaction time while in experiments 8 and 10 there was a slight increase in the reaction time. The reaction time may be increased or decreased in a given case. Under ether anesthesia, the response to the cyanide is less definite and would lead to the conclusion that ether depresses the irritability of the respiratory center to cyanide. Yet we have found that cyanide in sufficient dosage stimulates the respiration in dogs when it is depressed or paralyzed by ether.

The relation of the reaction time of the respiration to sodium cyanide to the complete circulation time in the rabbit and dog

	REACTION TIME	CIRCULATION TIME	R. T. / C. T.	C. T. / R. T.
Rabbit......................	3.97	4.71	0.84	1.19
Dog.......................	8.66	7.8	1.11	0.90

In the rabbit, therefore, the reaction time is approximately 84 per cent of the complete circulation time, whereas in the dog it is 111 per cent of the circulation time. If the reaction time to cyanide is used to determine the circulation time, the figure obtained for the reaction time in rabbits must be multiplied by 1.19, whereas in dogs the figure for the reaction time must be multiplied by 0.9 in order to arrive at the circulation time.

If one determines the average reaction time of an animal to sodium cyanide, the figure reached for the rabbit and dog will also be the complete circulation time within one second even though the correction which we have given above for these animals is not applied.

If we attempt to analyze the factors in the reaction time, we find the following:

1. Transport of the cyanide to the respiratory center.
2. Penetration of the center by the drug.
3. Latent period of the center.
4. Transmission of the nerve impulse to the muscles.
5. The latent period of the muscles.
6. The lag in the apparatus for recording the respiration.

The lag in the apparatus is a considerable fraction of a second. The time for the transmission of the nerve impulse and the latent period of the muscle is far beyond the accuracy of the method and need not be considered. The time required for the penetration of the center and the latent period of the center are factors which cannot be determined but must represent but a small fraction of a second. The only appreciable factor is the transport of the material to the center. Apart from this, the remainder of the reaction time is practically instantaneous. This further emphasizes the point made by Gasser and Loevenhart that reduced oxidation per se stimulates without invoking the production of lactic or other acid by reduced oxidation as the immediate stimulus.

SUMMARY

1. Lithium acetate, lithium benzoate and hexamethylene tetramine, because of the delicate tests for them in the blood, are admirably suited for the determination of the circulation time.

2. An improved technique for the determination of the circulation time is presented.

3. The average circulation time from the marginal ear vein to the opposite marginal ear vein in the rabbit is found to be 4.71 seconds. The average circulation time in the dog from

external jugular to external jugular vein is 7.8 seconds. The average reaction time of the respiration to sodium cyanide on injection into the marginal ear vein in rabbits is 3.97. The average reaction time of the respiration to sodium cyanide when injected into the external jugular vein in dogs is 8.66. A determination of the reaction time to sodium cyanide, either in the rabbit or dog, gives a figure which is within one second of the complete circulation time. In the rabbit the reaction time to cyanide is 84 per cent of the complete circulation time. In the dog the reaction time is 111 per cent of the circulation time. The effect of various factors on the circulation time and reaction time are discussed, such as the hour to hour and day to day variation, the relation to body weight, dosage, site of injection, pulse, anesthesia and bleeding.

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THE ACTION OF POTASSIUM SALTS ON THE MEDULLA AS SHOWN BY PERFUSION OF THE MEDULLA OF THE TERRAPIN (PSEUDOMYS TROOSTI) WITH POTASSIUM SALTS

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The ion-action of potassium is manifested most generally by depression of the central nervous system. Hooker (1) and Mathison (2), however, have brought forward evidence that potassium stimulates the medullary and spinal centers. In a series of experiments which were done to determine the action of various substances on the cardio-inhibitory center of the terrapin (Pseudomys troosti) potassium bromide was found to exert a marked influence on this center resulting in complete or partial inhibition of the heart. Subsequently the following series of experiments were performed to determine whether the potassium or the bromine ion was the causative agent. Results obtained indicate that the potassium ion is the causative agent since sodium salts and magnesium iodide were without effect; while the corresponding potassium salts produced inhibition.

The methods used were the same as reported in a previous article (3). In all cases except where noted otherwise 1:1,000 solutions were used The medulla was thoroughly washed out with amphibian Ringer solution which contained 0.03 per cent potassium chloride and which in no case influenced the medullary centers.

EXPERIMENTS

The following tabulated experiments are typical. In no case were sodium salts, even when used in 2 per cent strength,

found to exert any discernible influence.   In a series of experiments not reported here in detail, it was found that LiBr and the
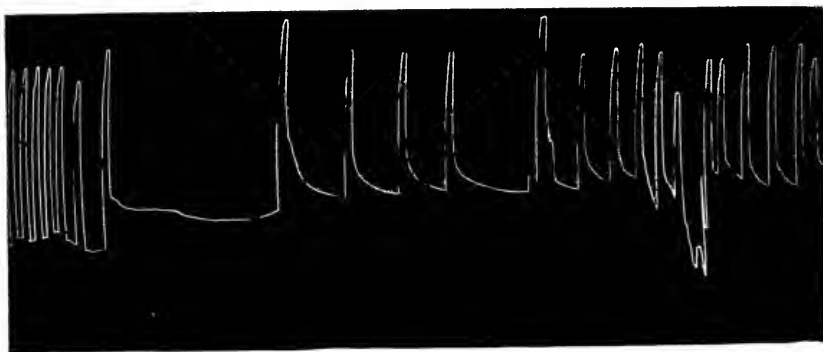
FIG. 1. SLOWING PRODUCED BY POTASSIUM BROMIDE
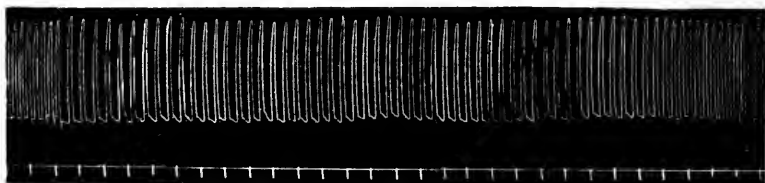
FIG. 2. SLOWING PRODUCED BY POTASSIUM CHLORIDE
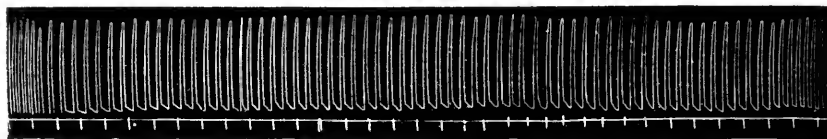
FIG. 3. SLOWING PRODUCED BY POTASSIUM IODIDE

halogens of ammonium all produce inhibition, hence sodium salts which were found inactive were used for control.   Magnesium iodide is also inactive.

FIG. 4. SLOWING PRODUCED BY POTASSIUM SULPHATE
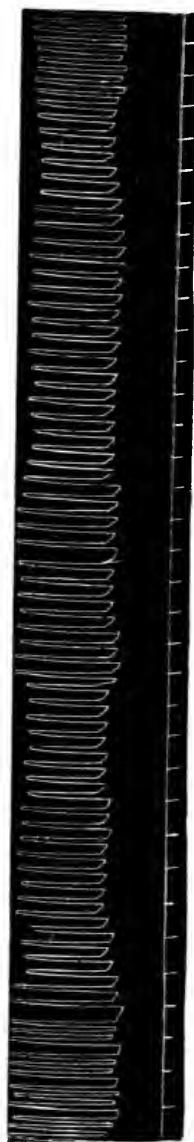
FIG. 5. SLOWING PRODUCED BY POTASSIUM FLUORIDE

FIG. 6. SLOWING PRODUCED BY POTASSIUM NITRATE

FIG. 7. SLOWING PRODUCED BY POTASSIUM TARTRATE

Fig. 8. Inhibition Produced by Potassium Hydroxide

Fig. 9. Slowing Produced by Potassium Bromate

Fig. 10. Inhibition Produced by Potassium Cyanide

Fig. 11. Slowing Produced by Potassium Thiocyanide

Experiment I.   July 27, 1921.   Pseudomys troosti

RATE	TIME	REMARKS
	minutes	
50		Normal with Ringer's
0	3	After KBr solution
42		After Ringer's
26	3	After KCl
4S		After Ringer's
49	12	After NaCl
4S		After Ringer's
24	3	After KI solution
32		After Ringer's
16	$3\frac{1}{2}$	After K_2SO_4 solution
3S		After Ringer's
22	5	After KBr
44		After Ringer's
24	$1\frac{1}{3}$	After KFl

It will be noted that in the above experiment only potassium salts influenced the center.

Experiment II.   August 3, 1921.   Pseudomys troosti

RATE	TIME	REMARKS
	minutes	
30		Normal with Ringer's
0		Stimulation of vagi
29		After Ringer's
14	$3\frac{1}{2}$	After KNO_3 solution
32		After Ringer's
0	$S\frac{2}{3}$	After $K_2C_4H_4O_6$
20		After Ringer's
0	3	After KOH
2S		After Ringer's
29		After NaBr
30		After Ringer's
2	5	After $KBrO_3$
31		After Ringer's
0	3	After KCN
29		After Ringer's
12	12	After KCNS

Experiment III

RATE	TIME	REMARKS
	minutes	
45		Normal
24	$2\frac{1}{2}$	KBr 1:1,600 solution
47		After Ringer's
49	7	After Br water
20	$4\frac{2}{3}$	After KCl solution
46		After Ringer's

Experiment IV

RATE	TIME	REMARKS
	minutes	
71		Normal
0	10	After KBr solution
68		After Ringer's
69	12	After NaBr 1:500
70		After Ringer's
0		Stimulation of vagus
66		Normal
68	15	After NaCl 2 per cent
0	11	After KBr solution

DISCUSSION

Potassium is characterized as a central nervous system depressant (4), (5). That it has another influence on the nervous tissue is indicated in the above experiments and in the results of Hooker and Mathison, both of whom state that potassium acts as an excitant.

This influence of potassium may be due to one of three causes:

1. Salt action.

2. Removal of the inhibitory influences of the cerebrum by depressing the cerebral cells.

3. Stimulation, irritation, or excitation of the nervous tissue.

The first, salt action, may be dismissed quickly as a causative agent since hypotonic, isotonic, or hypertonic solutions of sodium salts exert no discernible influence. We may conclude that salt action at the most is a negligible factor.

It may be assumed that potassium might depress the cerebral cells, removing the inhibitory influence which they exert on the medulla and in this manner allow the cardio-inhibitory center to function without restraint.   Were this the case we should expect prolonged perfusion to produce depression of the vagus center and hence cause the heart to assume a normal rate.   Prolonged perfusion, however, results in complete inhibition.   It is doubtful whether the vagus center of the terrapin has a normal tone, since section of the vagi is not accompanied by acceleration.

Since the ultimate results of excitation, irritation, and stimulation of the cardio-inhibitory center are the same it is difficult to state the exact influence of potassium, and at present we have no way to determine the exact action.

The potassium in the Ringer's solution has no effect since both the sodium and the calcium exert an antagonistic action.

That the anion does not exert any influence is proved by the fact that sodium salts containing the same anion as the potassium salts have no action.   Bromine water, likewise is without effect.

CONCLUSION

1. Potassium influences the cardio-inhibitory center of the terrapin (Pseudomys troosti) producing inhibition of the heart.

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SCIENTIFIC PROCEEDINGS OF THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

THIRTEENTH ANNUAL MEETING, HELD AT NEW HAVEN, DECEMBER 28–29–30, 1921

Edited by the Secretary, E. D. Brown

Observations on the Physiology of Vomiting. ROBERT HATCHER AND SOMA WEISS.

Experimental evidence is presented to show that afferent impulses from the stomach and from the heart pass up by way of the vagus and the sympathetic to the vomiting center after the administration of poisons which act upon these organs to induce emesis.

Both of these paths are also concerned in emesis induced by apomorphine and that induced by pilocarpine.

Some evidence is submitted tending to show that emesis is always a reflex act; impulses pass up to the vomiting center in the normal animal and that these normal impulses which are ineffective in causing vomiting in the normal animal do induce vomiting when the center is rendered hyperexcitable by apomorphine, strychnine or brucine and perhaps by toxins formed in disease.

Influence of Various Factors on the Excretion and Decomposition of Hexamethylenamine. P. J. HANZLIK AND FLOYD DE EDS. From the Departments of Pharmacology, Leland Stanford Junior University, San Francisco, and Western Reserve University, Cleveland.

The quantitative excretion of hexamethylenamine in human individuals was found to be rather variable, ranging from 32 to 82 per cent. The excretion lasted from twenty to thirty-three hours with 1-gram doses and about forty hours with 5-grams doses of the drug. The total excretion was uninfluenced by diuresis, but was augmented (up to 100 per cent) by the previous administration of bicarbonate, indicating that the acidity of the gastric contents is an important factor in the decomposition of the unexcreted portion.

Decomposition with liberation of free formaldehyde in tissues appears improbable, since only very low concentrations of formaldehyde dialyze and can be removed by aeration from neutral and slightly acid (pH 7 and 6.4), and none from alkaline, mixtures of hexamethylenamine and serum and plasma previously incubated for various periods at 38°.

Free formaldehyde was not demonstrable in the blood of animals

receiving large doses of hexamethylenamine intravenously. Accordingly, therefore, a rational basis for the alleged benefits of hexamethylenamine intravenously in the treatment of pneumonia and other specific infectious diseases is lacking.

Experimental Plumbism: Therapeutic Efficiency of Some Agents, and Comparative Toxicity of Certain Metals. P. J. HANZLIK, MARY McINTYRE (by invitation), AND ELIZABETH PRESHO (by invitation), Leland Stanford and Western Reserve Universities. Read by title.

Urinary Excretion of Salicyl after the Administration of Salicylates and Salicyl Esters. P. J. HANZLIK, FLOYD DE EDS (by invitation), AND ELIZABETH PRESHO (by invitation), Leland Stanford Junior University. Read by title.
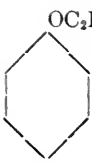
A Comparison of the Antiseptic Action of Various Aromatic Ethoxy and Hydroxy Compounds upon the Pneumococcus and the Gonococcus. A. D. HIRSCHFELDER AND L. J. PANKOW (by invitation). From the Department of Pharmacology, University of Minnesota.

When an ethoxy group is substituted for the methoxy on the quinolin ring of the hydroquinine molecule, it has been shown by Morgenroth and his collaborators that there is imparted to the substance ethylhydrocupreine a marked bactericidal and chemotherapeutic power which is specific against the pneumococcus. However, ethylhydrocupreine is too toxic for clinical use. No comparison has previously been made to determine whether, in simpler chemical compounds, the introduction of an ethoxy group would affect the antiseptic action of the substance. This question has been studied upon a series of aromatic compounds, comparing, wherever possible, the action of the ethoxy compound with the corresponding hydroxy compound. A pneumococcus (Rockefeller Type I) and a gonococcus were studied. Bactericidal action was determined by immersing a suspension culture in a weak (autoclaved) solution of the substances for the specified number of minutes, and then determining its viability by making a stroke culture on rabbit's blood agar. The agar was incubated upon a plate and growth on the agar was determined twenty-four and forty-eight hours later. The gonococcus was grown in Vedder's bouillon, and subsequently tested on starch bouillon and rabbit's blood agar.

The following substances were tested, + indicating growth of the cocci after being exposed to the drug for the period indicated, − indicating that the cocci did not grow.

1. Sodium salicylate 5 per cent, the Pneumococcus { + after 10 minutes / − after 60 minutes
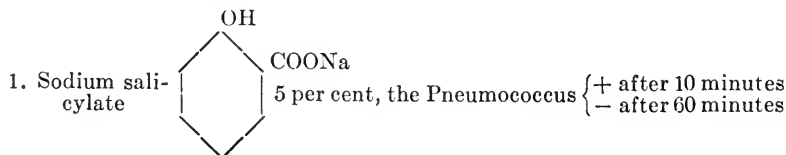
2. Sodium ethylsali-
   cylate

OC$_2$H$_5$
COONa
5 per cent Pneumococcus, + after 60 minutes

3. Sodium phenol-
   sulphonate

OH
SO$_2$ONa
5 per cent, Pneumococcus + after 2 hours
1 per cent, Gonococcus + after 30 minutes

4. Potassium phenetol-
   sulphonate
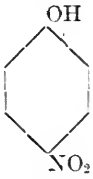
OC$_2$H$_5$
SO$_2$OK
5 per cent, Pneumococcus + after 2 hours
1 per cent, Gonococcus + after 30 minutes

5. Para amino-
   phenol
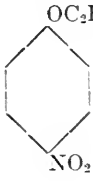
OH
NH$_2$
1 per cent, Pneumococcus + sometimes − sometimes
   after 30 minutes
1:1000, Gonococcus − in 10 minutes

6. Para phene-
   tidin
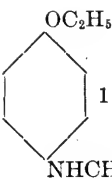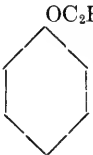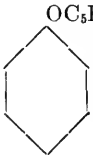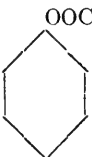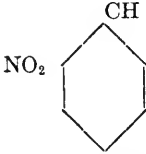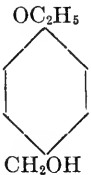
OC$_2$H$_5$
NH$_2$
1 per cent, Pneumococcus + sometimes − sometimes
   after 30 minutes
1 per cent, Gonococcus − after 10 minutes

7. Para nitro-
   phenol

OH
NO$_2$
1 per cent, Pneumococcus + after 10 minutes − after
   30 minutes
1:500, Gonococcus + after 10 minutes − after 30
   minutes

8. Para nitro-
   phenetol

OC$_2$H$_5$
NO$_2$
1:1000 (saturated solution) Pneumococcus + after 60
   minutes
1:1000, Gonococcus + after 60 minutes

OC$_2$H$_5$

9. Phenetidinethyl-
   alcohol

1:250 $\begin{cases} \text{Pneumococcus} + \text{after 30 minutes} \\ \text{Gonococcus} + \text{after 30 minutes} \end{cases}$

NHCH$_2$CH$_2$OH

OH

CH$_2$OH

10. Saligenin

2 per cent (in serum) $\begin{cases} \text{Pneumococcus} - \text{after 60 minutes} \\ \text{Gonococcus} + \text{after 30 minutes} \end{cases}$
2 per cent − after 60 minutes

OC$_2$H$_5$

CH$_2$OH

11. Saligenin ethyl
    ether

Saturated solution in 0.9 per cent NaCl (about
1:10,000) Pneumococcus. Results vary
8 repetitions − after 5 minutes
16 repetitions + after 30 minutes

OC$_5$H$_9$

CH$_2$OH

12. Saligenin iso amylether

Saturated solution less than 1:10,000
Pneumococcus − after 5 minutes
Gonococcus − after 10 minutes
Staphylococcus and Bacillus coli
both + after 30 minutes

OOCCH$_3$

CH$_2$OH

13. Acetyl saligenin

1:200 $\begin{cases} \text{Pneumococcus} - \text{after 5 minutes} \\ \text{Gonococcus} - \text{after 10 minutes} \end{cases}$

CH

NO$_2$ ⟨⟩ CH$_2$OH

1:200 Pneumococcus + after 30 minutes
1:200 Gonococcus − after 30 minutes
1:200 $\begin{cases} \text{Streptococcus} + \text{after 60 minutes} \\ \text{Staphylococcus} + \text{after 60 minutes} \end{cases}$

14. 1—nitro, 2—hydroxy, 3—phenyl carbinol

OC$_2$H$_5$

NH$_2$

15.

Streptococcus 1:100 + after 60 minutes
Staphylococcus 1:100 + after 60 minutes
Pneumococcus 1:100 +
Gonococcus 1:100 + after 30 minutes

CH$_2$OH

(1) Ethoxy (2) aminophenyl- (4) carbinol

The substances tested in this series ranged from the lipoid soluble amines to the water soluble salts of the aromatic acids; and in this series, in contrast to the quinine derivatives, there was no evidence of any superiority of ethoxy compounds over the corresponding hydroxy compounds. On the contrary, wherever there was any difference, whatever, the hydroxy compound was the more active. Further studies upon the derivatives of oxy quinoline are in progress and will be reported later.

Contribution to the Chemico-Pharmacodynamic Relationship of Atropine and Homatropine. DAVID I. MACHT. From the Pharmacological Laboratory, Johns Hopkins University.

The classical researches of Ladenburg on the structure of atropine and the synthesis of various tropeins led almost immediately to a wide therapeutic application of homatropine as a mydriatic. Inasmuch as the mydriatic action of atropine is known to be through the parasympathetic nerve mechanism of the eye, namely, the paralysis of the parasympathetic endings of the oculomotor nerve; it has been generally assumed that the mydriatic action of homatropine or tropinmandelate was of exactly the same nature. An examination of experimental data on the subject, however, gives no proof to support this assumption. In the present investigation, the author became interested in the pharmacology of homatropine in connection with a study of mandelic acid. This acid is closely related to benzaldehyde and indeed can be readily prepared from the latter by treatment with hydrocyanic acid and water. Inasmuch as the author has already shown that benzaldehyde possesses the antispasmodic or relaxant properties on smooth muscle which are exhibited by benzyl alcohol and certain benzyl esters, it was thought possible that the action of homatropine may be exerted, at least partially, directly on smooth muscle itself. A series of experiments tended to corroborate this view. In the first place, the action of homatropine on other parasympathetic nerve endings, such as the vagus terminals in the heart, is very much weaker than that of atropine. Whereas a small dose of atropine completely paralyzes the vagus endings in the heart, so that electrical stimulation, even of great intensity, fails to inhibit the heart beat, it takes about ten times as much homatropine to produce the same effect. In the second place, when such experiments on the vagus are performed it is interesting to note that injections of homatropine are followed by a fall in blood pressure and a vasodilatation which is obvious even to the naked eye when the intestines are inspected. In the third place, a comparative study of atropine and homatropine on uterine, intestinal and other smooth muscle in vitro, showed that the relaxant effect of homatropine was much greater than that of atropine. Furthermore, two other esters of mandelic acid which have been employed therapeutically were also found to exhibit marked antispasmodic effects on smooth muscle. These are antipyrin-mandelate, or *tussol* and eucain-mandelate, or *euphthalmin*. While antipyrin itself and eucaine itself

have very little effect on smooth muscle the mandelic acid esters of these substances were found to be markedly antispasmodic or depressant for that tissue. Finally, the author has prepared and studied the simple salts of tropic acid and mandelic acid themselves and found that whereas sodium tropate has little, or no effect on the contractions and tonus of smooth muscle, sodium mandelate exhibits a relaxant action and when used in strong solution (10 per cent), it was found to produce even a mild mydriasis when instilled into a rabbit's eye. The above data indicate fairly conclusively that the mydriatic effect of homatropine is not entirely due to a paralysis of the parasympathetic innervation but is probably, at least in part, to be explained by direct action of the drug on the muscle cells themselves. Further work, on the subject is in progress. This investigation is supported, in part, by a fund from the Research Council of the American Pharmaceutical Society. The author is investigating the properties of benzylmandelate.

Experimental Inquiry into the Narcotic Properties of Some Polyhydric Alcohols. D. I. MACHT AND GIU CHING TING (by invitation). From the Pharmacological Laboratory, Johns Hopkins University.

Following a comparative study of the toxic effects of alcohol, caffeine and nicotine on rats as indicated by the behavior in the circular maze, the authors began in the present research an inquiry into the narcotic properties of some polyhydric alcohols. White rats were trained in the maze so as to run from the periphery to the center in the quickest time without committing any errors. They were then injected with solutions of the substances studied and the effects of the drugs were noted repeatedly. Afterwards the following polyhydric or polyacid alcohols were examined: ethylene, glycol, glycerol, erythrite, arabite, mannite, dulcite, perseite and volemite. It was found that whereas the smallest effective dose of ethanol needed to produce a narcotic effect in the maze was 80 mgm. per 100 grams weight of rat, glycol produced the same effect after injections of 120 mgm. glycerine was also found to be narcotic but in doses of 160 mgm. per 100 grams weight. Narcotic or depressant effects were also noted after injections of erythrite, arabite, mannite and dulcite. The results of the experiments indicated that most of the polyhydric alcohols studied could produce a narcosis but the efficiency in this respect decreased with the number of hydroxyl groups present in the molecules. Fuller details will appear in the Journal of Physiology.

Studies in the Toxicity of Aliphatic Alcohols. J. C. MUNCH (by invitation), AND E. W. SCHWARTZE.

This study was undertaken for the reason that very few useful data on the comparative oral toxicity of the aliphatic alcohols exist. The size of the dose required to produce a given effect is larger in the case of the oral method of administration than in that of the usual subcutaneous and intravenous procedures, and delayed effects are more

easily produced. The rate of absorption in the oral method appears to be more uniform than that obtained with the subcutaneous method, and is not discontinuous as is usually the case with intravenous experiments.

The oral method was used in these experiments because consideration of the effects of protein precipitation, believed to be obtained with the use of the intravenous method, is thus avoided. The absorbing surface is greater than with the subcutaneous method, permitting the administration of doses of dilute alcohol by mouth, the subcutaneous administration of which, in the same concentration, would be impracticable. If the results of the intravenous and subcutaneous experiments reported by other investigators are a fair index of the usefulness of such methods, the oral method of administration seems just as useful, if not more so, for the purpose of toxicity studies.

It has been assumed that the administration of hirudin protected animals from the intravenous precipitation of blood and serum proteins by alcohol. Ordinarily, clotting of blood is regarded as a process different from the precipitation of proteins by simple chemical agents. Very definite rings of precipitated material (presumably proteins) have been obtained by stratifying hirudinized centrifuged rabbit serum with hirudinized 25 per cent ethyl alcohol. With lower concentration of alcohol the tests were less positive. It cannot be assumed, therefore, that the use of hirudin is necessary, or that it prevents pulmonary embolism from the administration of alcoholic solutions.

The preliminary results show that the oral toxicity increases with the increasing molecular weight, and that the range between the lethal and the narcotizing doses diminishes for an homologous series. Although numerous data have been obtained, they are not considered sufficient for establishing the theoretical relations of the alcohols to one another.

Further Studies on the Action of Mercury. WILLIAM SALANT AND NATHANIEL KLEITMAN (by invitation).

Intravenous injections of mercury in the form of the succinate were given to cats under urethane anesthesia. One milligram of the metal per kilo administered in five minutes at intervals of thirty to thirty-five minutes, produced essentially the same effects as when the salt was introduced much more rapidly. Blood pressure fell, and inspection of the heart and myocardiographic records showed heart-block and delirium cordis after the injection of 4 to 5 mgm. per kilo. Paralysis of respiration and arrest of the heart occurred after the administration of 8 to 13 mgm. mercury per kilo, indicating that the rate of injection exerts only a moderate effect on the toxicity of mercury. When preceded by small amounts of sodium citrate, milligram doses of mercury caused a very considerable increase in respiration, which often lasted several minutes after the salt of mercury was injected. Repeated injections of mercury, however, produced the usual depressing action, though preceded by citrate. When mercury succinate was given intramuscularly or was

introduced into the small intestine, the effects were similar to those observed after slow intravenous injections. Circulatory depression and cardiac irregularity were sometimes observed sooner after duodenal than after intramuscular injections, the doses being the same in each case. Small doses of adrenalin injected intravenously hastened the onset of cardiac irregularity observed after the injection of mercury.

The Pharmacology of Citrates. WILLIAM SALANT AND NATHANIEL KLEITMAN (by invitation).

The action of sodium citrate was studied by intravenous and intramuscular injections in cats and dogs, and by perfusing the isolated heart of the turtle. Doses of 30 to 45 mgm. of the salt per kilo given intravenously to cats, produced a prompt fall in blood pressure and stimulated respiration, when the injection was rapid. If the salt was given slowly, little or no disturbance of the circulation and respiration occurred. Larger doses caused depression of the circulation as well as respiration, but in either case recovery was observed within a few minutes. The cat's heart in situ was depressed by the citrate as shown by records obtained with the myocardiograph.

In experiments on the isolated heart of the turtle, which was perfused with different concentrations of sodium citrate, cardiac depression was produced by $M/300$, and in some cases even by $M/1000$ of the salt.

Intravenous injections given very slowly to cats sometimes caused depression of the respiration after a sufficient quantity was introduced. Intramuscular injections of citrate indicated slow absorption. The experiments on dogs showed that in these animals doses of 50 to 70 mgm. citrate per kilo, given intravenously produced a moderate rise of blood pressure and stimulated respiration. Larger doses caused paralysis of the heart and of respiration.

Studies on the mechanism of citrate action were made by testing the effect of different concentrations of the salt, and by comparing the action of oxalate, tartrate and citrate on the isolated heart of the frog and of the turtle. The results indicated that the action of the three salts was not due to precipitation of calcium. The citrate was considerably more toxic than the oxalate or tartrate, and its action was proportional to the concentration.

The Effects of Sodium Fluoride upon the Calcium Balance and the Calcium Content of the Blood. GUY W. CLARK (by invitation). From the Department of Biochemistry and Pharmacology, University of California, Berkeley.

Experimental work with a number of the so-called calcium precipitants (citrates, malates, phosphates) has been previously reported by the writer.

Sodium flouride was selected because of the insolubility of calcium fluoride (0.0016 gram per 100 cc. H_2O at 18°C.). It might be expected that the absorption of such a substance would materially lower the calcium of the blood and also affect the calcium balance.

Rabbits on a calcium-rich diet (alfalfa leaves, barley, carrots) were used as experimental animals. After a control period (fourteen days) daily subcutaneous injections of 2 per cent sodium fluoride were given for twenty-one days. The amounts injected daily varied from 10 to 40 mgm. per kilo for different animals. The slightly negative balance during the last days of the experiments is undoubtedly due to the lowered food intake. The calcium content of the blood is decreased about 10 per cent.

On the Causation of Experimental, Anaphylactic Hemorrhages in the Stomach. JOHN AUER. From the Department of Pharmacology of the St. Louis University School of Medicine.

All the experiments were carried out on guinea-pigs sensitized by the subcutaneous injection of 0.5 cc. normal horse serum; the incubation period was generally twenty weeks or more; the reinjection was usually intraperitoneal, 2 to 3 cc. horse serum being employed; a few times the subcutaneous route was chosen for reinjection. Most of the animals died, or were killed by medullary puncture within four hours after reinjection; in a few cases forty-eight hours more or less elapsed. Autopsies were always performed at once after cessation of respiration. The material consists approximately of sixty animals; about one-half served as normal controls or serum controls. The material for microscopic study (the entire stomach) was pinned out in Orth's fluid after slitting along the greater curvature. After fixation was complete, pieces were cut from the antral and preantral region, the middle third and the fundus, the three or four pieces being mounted in one paraffin block for convenience of comparison. The main new results are as follows:

The earliest lesions observed consisted of short, superficial linear or starshaped, brownish erosions of the mucosa in the middle third of the stomach; they were located on both anterior and posterior surfaces, and generally much nearer the greater curvature than the lesser one. Careful inspection showed no sign of blood either in the stomach contents or in the erosions themselves.

In the next stage hemorrhages of peculiar structure and variable extent were noted. In typical instances the hemorrhage was composed of small, flattened, black, discoid masses of blood more or less superimposed one upon the other so that sometimes the hemorrhage looked like a miniature compressed bunch of grapes. On removal of the hemorrhages by washing in Ringer solution, linear irregular erosions were disclosed; these were always fewer in number and smaller in extent than was expected from the size of the hemorrhage. The bottom of the erosions showed numerous minute red-brown dots. At times flat surface erosions without any bleeding points were observed adjacent to an area of hemorrhage; this flat eroded patch looked brownish, contained large numbers of pin point, brownish dots and was covered by a whitish layer of mucus.

After forty-eight hours only fairly deep linear erosions with pouting lips were generally found.

The microscopic examination showed that the hemorrhages were always in the mucosa; none were ever seen in the submucosa or in the muscular coats. The erosions never extended below the muscularis mucosae, but only up to this muscle sheet.

Local extreme dilatation of many but not all mucosal blood capillaries was very common in the middle third of the stomach, but was not generally observed to the same degree in the preantral and antral regions. These local capillary dilatations were always especially noticeable near areas of hemorrhage. These hemorrhages occurred not only in the lower part of the mucosa but also near the free surface. The arteries and veins in the submucosa showed markedly dilated and constricted sections, so that they looked irregularly beaded.

It is believed that the following mechanism explains the observations described above: the erosions and hemorrhages are the result of a local auto-digestion. This auto-digestion is localized because it only occurs in asphyctic areas of the mucosa. The local asphyxia is caused by a decrease or stoppage of the blood circulation in the capillaries of that region. The focal capillary circulation is deficient because apparently travelling peristaltoid contractions in the arterioles, veins and even capillaries impede or temporarily abolish the transit of blood in these areas. These peristaltoid contractions also would explain the peculiar structure of the hemorrhages alluded to above. Further details and experimental evidence will be submitted in the final paper.

It should be pointed out that the mechanism described above, or part of it, offers a ready explanation for the occurrence of capillary hemorrhages during various diseases in many different structures, for example, the intestine, the muscles, the lungs or the skin; it may be also of service in explaining the causation of the various types of erythema seen in the acute infectious diseases.

Effect of Caffeine on Intestinal Movements. O. H. PLANT AND C. REYNOLDS (by invitation).

Isolated pieces of rabbits' intestine (duodenum, ileum) contracting in oxygenated Tyrode's solution, show distinct increase in amplitude of contractions and in "tone" with 1:40,000 caffeine. When higher concentrations (1:20,000 to 1:10,000) are applied, the increase in "tone" is more marked.

In experiments on unanesthetized dogs (10 to 12 kgm.) with Thiry-Vella loops of ileum, in which the intestinal contractions were recorded by means of a rubber balloon filled with water and connected with a Brodie bellows-recorder, the tone of the loop, the frequency of peristaltic waves and the height of the individual rhythmic contractions were increased by subcutaneous doses of caffeine of the order of 5 mgm. per kilogram.

These effects of caffeine are not prevented by atropine.

Experiments with Aconitine and Barium on the Isolated Heart. WILLIAM
SALANT AND NATHANIEL KLEITMAN (by invitation).

In perfusion experiments with the isolated heart of the frog and of the
turtle, it was found that dilute solutions of barium chloride (1 part
barium in 10:100,000 parts Ringer's) produced slight depression or was
without any effect. The depressing action of barium was especially
pronounced when the heart was previously exposed to the action of
aconitine. The reduction in cardiac efficiency appeared promptly
after perfusion with barium chloride was begun, and lasted until it
was discontinued. Force as well as frequency was decreased by the
barium chloride under these conditions. In some experiments one
treatment with aconitine was sufficient to cause depression after all
subsequent perfusions with barium chloride.

*Summary of Results from the Treatment of Human Trypanosomiasis
with Tryparsamide.* LOUISE PEARCE AND WADE H. BROWN. From
the Laboratories of the Rockefeller Institute for Medical Research,
New York.

The initial observations upon 77 patients suffering with trypano-
somiasis caused by *Tr. gambiense* and treated with tryparsamide
(the sodium salt of N-phenylglycineamide-p-arsonic acid) have been
fully reported elsewhere (*Jour. Exper. Med.*, xxxiv, No. 6, Supple-
ment No. 1). Subsequent observations of most of these patients
during the first year of treatment are now available.

Among the 21 previously untreated, early cases treated with single
doses of tryparsamide of 1 to 5 grams, there has been 1 additional
relapse, 168 days after treatment and 1 probable relapse in a patient
in whom no trypanosomes were demonstrated, but whose spinal fluid
showed an increase in cell content. Six patients are reported as nega-
tive, 75 to 292 days after treatment.

Fifteen patients who relapsed after single dose treatment were
re-treated with 1 and 2 courses of tryparsamide, each course consisting
of 1 to 10 doses of 1 to 5 grams, administered intravenously at
semi-weekly, weekly, and bi-weekly intervals. No relapses have
been detected during observation periods of 68 to 299 days.

There were 16 previously untreated, early cases who received from
2 to 8 doses of tryparsamide, ranging from 0.3 to 5 grams, administered
intravenously at weekly, fortnightly and monthly intervals. Of
these, 6 relapsed in from 38 to 110 days, while no return of trypano-
somes has been demonstrated in 10 patients during a period of 151 to
250 days.

Twenty-one previously untreated, advanced cases have been treated
with 1 to 4 courses of tryparsamide, each course consisting of 2 to 10
doses of 0.7 to 5 grams, administered intravenously. Examination
of the spinal fluid before treatment revealed a marked increase in the
number of cells which ranged from 15 to 387 per cm. There have
been 3 relapses in 89, 179, and 317 days and 18 non-relapses in 78 to
380 days. Examination of the spinal fluid in both relapsed and non-

relapsed cases shows a marked decrease in the cell content, the highest count being 22, 5 counts of from 6 to 10, and 15 counts of 0.9 to 5 cells per cm.

Of 7 advanced patients previously treated with a variety of drugs and transferred to tryparsamide therapy, 2 extremely advanced cases have died. The other 5 patients have received 1 to 4 courses of tryparsamide, each course consisting of 2 to 8 doses of 2 to 7 grams. The initial cell counts of the spinal fluid in these 7 patients ranged from 32 to 572 cells per cm. The last examination made, after 35 to 385 days showed cell counts of from 0.9 to 25 cells per cm.

These observations are in accord with the earlier results obtained with tryparsamide. The outstanding features of its use in human trypanosomiasis are its trypanocidal activity and its effect upon the spinal fluid as evidenced by the marked reduction in cell content.

Rate of Excretion of Arsenicals. A Factor Governing Toxicity and Parasiticidal action. CARL VOEGTLIN AND J. W. THOMPSON (by invitation).

The comparative rate of excretion of the arsenic of various types of arsenicals was studied with a view of correlating the rate of excretion with the toxicity and parasiticidal action. It was shown, in a general way, that both toxicity and parasiticidal action increase with a reduction in the rate of excretion of the arsenic and vice versa. Marked variations in the excretion of the arsenic of a given drug are observed in different individuals of the same species (rats). This probably accounts for the well known individual differences in the toxicity and parasiticidal action of a given arsenical.

Effect of Ligation of the Ureters or Bile Duct upon the Toxicity and Trypanocidal Action of Arsenicals. CARL VOEGTLIN, HELEN A. DYER (by invitation), AND DOROTHY W. MILLER (by invitation).

Ligation of both ureters causes a great increase in the toxicity and trypanocidal action of those arsenicals (pentavalent) which in normal animals are rapidly excreted with the urine. On the other hand it was found that ligation of the ureters does not materially influence the toxicity and trypanocidal action of those arsenicals (arsphenamine, trivalent arsenoxides), which under ordinary conditions exhibit a low rate of urinary excretion. Ligation of the common bile duct increases the trypanocidal action of arsphenamine.

The Significance of the Marked Variations in the Toxicity of Undissolved Arsenious Oxide. ERICH W. SCHWARTZE, Pharmacological Laboratory, Bureau of Chemistry.

During a study conducted in the Bureau of Chemistry it was found that marked and significant variations occur in the toxicity and potency of undissolved arsenious oxide. This lack of uniformity is due to the difference in the average size of the particles constituting each preparation. One finely-divided preparation of arsenious oxide consumed by

rats in their food proved to be five times more toxic than one of the coarse preparations. One finely-divided preparation administered by a stomach tube to rabbits was eight times more toxic than another, but coarser, preparation. In the case of chickens, however, the differences observed were not so great, because of the fact that chickens have gizzards in which, presumably, the larger particles are ground. The highly toxic preparations examined were made in a ball mill and in their toxicity closely approached dissolved arsenious oxide.

For convenience of technique and other reasons the potency of large crystals of arsenious oxide was studied on cats by means of the emetic reaction and calculated on the basis of the concentration in the diet. To produce emesis, 420 times as much undissolved arsenious oxide, in the form of crystals from 2.5 to 5 mm. thick, as dissolved oxide was required.

The significance of these observations lies in their bearing upon the alleged proof of the habituation of man and animals to arsenic. Curiously, experimenters have been able to supply an apparent proof of this assumption in a supposedly conclusive manner only by using undissolved arsenious oxide (Cloetta, Hausman). A review of the literature upon this subject and upon "arsenic" eating, however, shows that the fineness of the particles administered has not been taken into consideration. Moreover, with one exception, even the oral toxicity of undissolved arsenious oxide has been studied without any reference to the size of the particles constituting the preparations administered.

Although detailed discussion of the voluminous literature is out of place in a preliminary publication, it may be stated that no positive proof has been presented by the authorities (Hausman, Cloetta, Danger and Flandin, Joachimoglu, Knapp et al., Schäfer and von Tschudi) to show that habituation exists in man or other higher animals. It is significant, though not certainly indicative of the opposite contention, that some investigators (Brouardel, Morischima, Hausman, O'Kane et al., and Sollmann) have failed to obtain habituation.

At present the proof of habituation to undissolved arsenious oxide rests chiefly upon a fallacious premise. An experimental proof of tolerance to either the dissolved or undissolved arsenious oxide is wanting.

Experiments Dealing with Certain Effects of Quinidine Sulphate on the Dog's Heart. ALFRED E. COHN AND ROBERT L. LEVY. From the Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.

Oral administration of quinidine sulphate to patients with auricular fibrillation serves, in about 50 per cent of cases, to restore the normal cardiac rhythm. A study of the pharmacologic action of this drug is important in its relation to clinical therapeutics and affords a means for approaching the investigation of the disturbed physiologic mechanism which is at the basis of fibrillation of the auricles.

In a preliminary communication we have shown that intravenous injection of quinidine sulphate exerts the following effects on the cardio-vascular apparatus of the dog:

1. *Heart rate* was inconstantly affected. Acceleration, retardation and fluctuation were observed at different times.

2. *P–R (conduction) time.* There was usually slight prolongation of conduction of the impulse through the A–V bundle. Shortening was observed once.

3. *T-wave of electrocardiogram.* Reversal in the direction of the deflection or of increase in voltage of the original wave was com-monly seen.

4. *Threshold for the production of auricular fibrillation by Faradization.* In about half of the experiments this was slightly raised. In the remainder, no effect was noted.

5. *Blood pressure.* In all of the animals a striking fall in arterial pressure followed the injection, the extent of the fall depending in a measure on the amount of drug given. There was generally partial, but never complete, return to the former pressure level.

6. *Degree of Muscular contraction.* This was recorded by means of the Roy and Adami myocardiograph. The recent roentenographic studies of Eyster and Meek furnish evidence that the use of a linear measurement of muscle shortening obtained by the use of this apparatus may be employed as a reliable indication of volume output. There was *invariably* an increase in the height of the stroke recorded by the lever, the increase ranging from 16 to 162 per cent. This effect is of particular significance since derivatives of cinchona have always been regarded as depressants of the contractile function of the heart. A point was always reached after which further introduction of the drug caused diminution in volume output. The augmentation in ventricular contraction occurred synchronously with the fall in blood pressure, but always persisted after partial restoration of the blood pressure level.

In order to ascertain whether the increase in cardiac contraction was directly associated with the lowered arterial pressure, a series of ex-periments was done in which a fall in pressure was induced by other means, namely, hemorrhage and histamine. The effect on the height of contraction differed significantly from that observed after quinidine in that (*a*) results were inconstant; sometimes an increase was seen, in other experiments no change was observed. (*b*) When augmentation in cardiac output occurred, it was usually of brief duration, although the blood pressure rarely returned to its original level. (*c*) The increase by quinidine was due to greater muscle shortening, not to diastolic relaxation as was sometimes the case after histamine or hemorrhage.

Mode of death. There was, with the exception of one animal which died with ventricular fibrillation, progressive slowing of the heart, sometimes with occurrence of sino-auricular block. The auricles as a rule, ceased before the ventricles. In the final curves were seen isolated, orderly ventricular beats.

Lethal dose. This was extremely variable (mgm. per kgm.). In general, the greater the fractionation of dosage, the greater was the amount of drug necessary to cause death.

More recently we have investigated the rate of conduction of the excitation wave through the auricular muscle of the heart in situ. There was usually a delay in the rate of travel of the excitatory process. We have also made studies of the refractory period of the natural heart, i.e., with vagi intact and with no attempt to control rate. The effect of quinidine on the refractory period was variable, in that in a certain number of experiments it was lengthened, in some shortened and in others unchanged. This result was to be anticipated, first on the basis of clinical experience, and second on the theory that circus movement is the mechanism underlying fibrillation. Inasmuch as the rate of conduction of the impulse through auricular muscle is usually prolonged by quinidine, induction of the cessation of auricular fibrillation by this drug depends upon lengthening of the refractory period. The variability with which this effect can be produced in dogs coincides with the inconstancy of the results obtained in the clinic in the treatment of auricular fibrillation with quinidine. It is possible that restoration of normal rhythm is more likely to ensue when the refractory period is initially short, thereby making possible significant lengthening by the drug.

Further studies of the action of quinidine are in progress. These deal with the effect on the refractory period and on the rate of the conduction, of impulses, when the rate of the heart is maintained at constant levels, and when the vagus tone is varied, either by stimulating the nerve or abolishing its effect.

Observations on the Action of Cinchophen in Human Subjects with Normal Nucleic Acid Metabolism. G. P. GRABFIELD (by invitation), AND J. H. PRATT.

The uric acid excretion of six patients on a purin-free diet was studied and found to vary within rather wide limits The nucleic acid metabolism of these subjects was apparently normal. The endogenous level of these cases varied within 100 mgm. output per day, the average being about 350 mgm. per day.

The administration of cinchophen was found to increase the output. However, this increase over the endogenous level never continued for more than three days and usually did not persist beyond the second day. In at least one case cinchophen produced no increase in excretion. If the administration of this drug was discontinued after two days (the dose used was three grams a day divided into three or six doses), the endogenous level was resumed, but if the administration was continued for five days there occurred a compensatory fall in output which sometimes more than counterbalanced the initial rise.

We could adduce no evidence that cinchophen invariably "sweeps out" the uric acid from the body in normal individuals.

Metabolism in Chloroform Poisoning. Frank P. Underhill and Robert Kapsinow (by invitation). Read by title.

A Quantitative Study of the Toxicity of Phenol Derivatives for Strongylid Larvae. W. H. Schultz. Read by title.

The Effect of Certain Drugs upon Faradic Sensibility. Worth Hale and G. P. Grabfield (by invitation).

The effect of diethyl barbituric acid, antipyrine, acetphenetidin and acetyl salicylic acid upon the human sensory cutaneous threshold was determined according to the method of Martin (Am. Jour. Phys., 1908 to 1914).

Diethyl barbituric acid and its sodium salt were given by mouth in doses ranging from 0.2 to 0.5 gram and the threshold determined at half-hour intervals for one and a half hours after the drug was taken. When the subjects smoked during the morning of the experiment, the threshold followed closely the normal threshold curve of smokers. In non-smoking experiments, no marked change was observed although there was a clear tendency for the threshold to be higher than the non-smoking normal threshold, thus furnishing some evidence that tobacco tends to maintain the threshold at the normal point.

In the group of antipyretic sedatives, studied in the same way with the exception that the subjects did not smoke, antipyrine and acetphenetidin caused a very distinct rise in the threshold. This is more prompt after antipyrine, being at its maximum in one-half hour but the effect of acetphenetidin is more prolonged and reaches an equally high level one and a half hours after its administration, which possibly coincides with its slow decomposition into paramidophenol. Acetyl salicylic acid apparently has no effect upon the threshold either to raise or to lower it during the period of observation.

The Toxicology of Hydrogen Sulphide. Howard W. Haggard (by invitation).

Hydrogen sulphide when inhaled does not form a compound with the hemoglobin. It does not combine with the sodium of the blood. It is transported as the dissolved gas. Blood has the property of rapidly oxidizing H_2S. If Na_2S is injected intravenously it is immediately hydrolyzed with the liberation of H_2S. Inhalation of H_2S kills in concentration of 7 parts in 10,000, but only after the lapse of many hours and then through lung edema. Concentrations of 8 to 20 parts produce extreme hyperpnea followed in a few minutes by death in apnea vera. In higher concentrations (over 20 parts in 10,000), death occurs almost instantaneously from respiratory paralysis. The respiratory stimulating action of H_2S is apparently upon the vagal endings in the lungs. The convulsions following H_2S are not entirely of cerebral origin as has been supposed for they occur in spinal cats. H_2S slows the heart through vagal inhibition.

Observations on the Pharmacological Action of Quinidine. J. V. Law-
rence (by invitation), Alfred Friedlander (by invitation) and
D. E. Jackson. From the University of Cincinnati College of
Medicine.

When quinidine sulphate is injected intravenously into a normal
animal (dog), the most striking effect produced is a fall in blood-
pressure. With small doses this is of moderate extent and tends to
disappear in a few minutes. Larger doses produce a greater fall which
shows a much greater tendency to last. This fall is apparently some-
times due partly to slight weakening and dilatation of the heart. We
believe, however, that a peripheral action of the drug on the arterioles
or capillaries, or both, is very important in determining the action of
the drug on general systemic blood-pressure.

We have repeatedly seen an immediate acceleration of the heart
beat, together with a marked increase in amplitude, and apparently in
the strength, of the right auricle as shown by the myocardiographic
tracing, when doses of 5 to 15 mgm. of quinidine sulphate were suddenly
injected into normal animals. This strengthening of the auricles is
more in evidence than is that of the ventricles, which also occasionally
are apparently slightly stimulated by small doses. The fall in systemic
pressure, however, obscures the earliest action on the ventricle some-
what. Very large doses slow and weaken the whole heart.

We have produced cardiac irregularities by the injection of barium,
digitoxin, veratrine and aconitine. In general, it may be said that
these irregularities may, while in the early stages, be frequently cor-
rected and the heart beat changed to normal for a longer or shorter
period of time. This, however, is usually transient, as the drugs here
used to produce the irregularity are lasting in their actions, while the
immediate regulating effect of the quinidine, as a rule, soon passes
off. This regulating action apparently affects the ventricles as well as
the auricles, although it may well be that the regulating influence is
primarily exerted on the auricles, which may have been fibrillating or
merely in a flutter. In the later stages of the irregularity produced
by either of these four drugs, quinidine has no power to restore the
normal beat to the heart. In these cases, it is to be presumed that the
four drugs used to produce the irregularities effect this result by a
direct stimulating action of the muscle fibers of the heart. Conse-
quently we might expect the quinidine also to act on the muscle fibers
in an opposite manner. We noted in a few instances that when the
irregularity produced by these drugs was very marked, but with the
pressure still high, the sudden injection of a dose of quinidine (15 mgm.)
may occasionally produce sudden death by stoppage of the heart. We
have opened the chest quickly in these animals and found either no
delirium cordis, or if any fibrillation was present, it was very feeble and
had evidently been greatly opposed by the action of the quinidine. We
have also noted that quinidine tends to counteract the early develop-
ment of delirium cordis under poisonous doses of such drugs as digi-
toxin or aconitine.

We have produced heart-block by injecting a mixture of 50 per cent each of chloroform and alcohol into the region of the auriculoventricular septum by means of a very fine hypodermic needle. In such hearts, when fibrillation or flutter of the auricles has been produced by Faradization, we have found that quinidine sometimes restores the normal beat.

The endings of the vagi in the heart are not paralyzed by quinidine, as has been supposed, but on the contrary these endings remain active until the heart ceases to beat as the result of enormous injections of quinidine. Indeed, the cardiographic tracings of the auricle in normal dogs rather indicate that the vagus has a little greater inhibitory action on the auricle after a few injections of quinidine have been given than would have been found without the quinidine. These results have been checked up by giving a small dose of atropine after the quinidine, but at a time when the after-effects (slow recovery) of vagus stimulation were still present in the auricle. The atropine then causes a very prompt disappearance of the slow and feeble beat of the auricle.

Clinical Observations on Quinidine. R. W. SCOTT.

Clinical and electrocardiographic studies of the action of quinidine sulphate were made in the following cardiac disorders: Auricular fibrillation, auricular flutter, paroxysmal tachycardia of auricular origin, and ventricular paroxysmal tachycardia with retrograde cardiac mechanism during attacks.

In patients without circulatory stasis the drug was readily absorbed as indicated by a definite effect on the electrocardiogram in from thirty minutes to one hour after administration by mouth. The first effect noted in both flutter and fibrillation was a retardation of the auricular rate. In one case of fibrillation an auricular rate of 530 per minute was slowed to 380 three hours after 0.6 gram quinidine. Similarly a case of flutter with auricular rate of 330 was given 0.5 gram quinidine and two hours later the auricular rate was 270 per minute.

As the auricle slowed in both flutter and fibrillation, the ventricle increased, but never to the point of a 1:1 response in the cases thus far observed.

The dosage of quinidine necessary for the restoration of a normal mechanism varied considerably, but in some cases a relatively small amount was sufficient. For example a case of fibrillation was changed to a normal mechanism with three doses of 0.2 gram and a case of flutter was converted to normal with a total dosage of 1.5 gram.

In addition to the remarkable effects of quinidine on the auricle in fibrillation and flutter due, as Lewis believes, to the prolongation of the refractory period, the drug also appears to exert a definite depressing action on both the auricle and ventricle. This was illustrated by its prompt action in a case of auricular paroxysmal tachycardia, and in one case of tachycardia of ventricular origin, studied over a period of eight months. On several occasions during this time quinidine stopped the paroxysms with restoration of a normal mechanism in from thirty minutes to one hour after the administration of 0.4 gram by mouth.

Further evidence of the depressing action of the drug on the heart was suggested by the clinical course in two fatal cases after conversion from fibrillation to normal. One of these patients received a total of 2 grams over a period of forty-eight hours and shortly after the appearance of a normal mechanism, he developed acute distress with marked distension of the veins of the neck, and died in a few minutes. Extreme dilatation of the right auricle was demonstrable by percussion, and at autopsy both auricles were markedly dilated. No emboli were found.

From the observations thus far made the following conclusions appear warranted:

1. Quinidine sulphate is absorbed in from thirty minutes to one hour after the administration of therapeutic doses by mouth.

2. The first effect in flutter and fibrillation is a retardation of the auricular rate. Later a normal mechanism is established in a certain percentage of cases.

3. The drug is a cardiac depressant and for this reason its use in patients with definite myocardial disease is not without serious danger unless carefully controlled.

4. Its therapeutic value is suggested in controlling certain cardiac irregularities due to ectopia foci of impulse formation.

A Note on the Action of Silver Salvarsan. D. E. JACKSON AND J. V. LAWRENCE (by invitation). From the Department of Pharmacology of the University of Cincinnati Medical College.

Small injections of silver salvarsan cause small, transient falls of the systemic blood-pressure. We are of the opinion that the action of silver salvarsan is not so depressant to the circulatory organs as a whole as is salvarsan. In fact, at times a slight prolonged rise in pressure appears to follow the primary fall after silver salvarsan. And repeated, small injections of the drug do not reduce the animal's vitality so quickly as does salvarsan. From this standpoint, it would appear that the silver compound is safer for intravenous injection than is the older drug.

But little action is, as a rule, exerted on the bronchioles by silver salvarsan, but occasionally there is produced a slight contraction. This can readily be overcome by adrenaline.

In this work, we have been primarily interested in the action of the drug on the lung circulation. We find that very small doses cause some immediate rise in the pulmonary pressure. This rise is sudden in its appearance and lasting in character. In no way, by drugs or otherwise, have we been able to satisfactorily overcome this pulmonary complication. The action here is apparently identical with that of salvarsan. And large doses produce a profound rise in the pulmonary pressure, our tracings showing an increase of fully 100 per cent. This action on the pulmonary vascular area is apparently always produced in some degree when any dose of the drug, no matter how small, is injected into the systemic veins. It is evidently due to a precipitation

of the compound in the blood, and the very fine particles thus formed are caught in the pulmonary capillaries. The exact nature of compound formed when the drug is precipitated is not yet clear, and we have suspected that the silver enters some soluble organic combination in which form it rapidly circulates about and exercises some mild stimulating action on the circulation, either directly on the arterioles, or else by a mild stimulation of the vasomotor center in the medulla. With large doses, the rise in pulmonary pressure rapidly reaches the maximum limit. We have found that injection of the drug by way of the portal circulation leads to a deposition of the precipitates in the liver capillaries, and that under these circumstances the pulmonary blood-pressure scarcely undergoes any change whatever. We have been able to show that after the drug has been repeatedly injected into the branches of the portal vein without affecting the pulmonary pressure, a small dose of the drug injected into the femoral vein will promptly cause a rise in the pulmonary vessels. Further we have found that if the pulmonary pressure be greatly raised by femoral vein injections of the drug, the systemic pressure meanwhile remaining high, then the intravenous injection of a dose (10 mgm.) of quinidine sulphate will produce a great fall in systemic blood-pressure, but will cause only a very slight decrease in the pulmonary pressure. Here it would appear that the quinidine lowered the systemic pressure very largely by peripheral dilatation of arterioles or capillaries, but that in the pulmonary area, the capillaries of which were plugged up by the precipitate of silver salvarsan, the quinidine could not, or does not act, and the quinidine did not weaken the right heart sufficiently to produce a fall in the pulmonary pressure.

The Influence of Veratrine and Epinephrine on Skeletal Muscle. THEO-PHILE K. KRUSE, University of Pittsburgh.

On account of conflicting reports, tests were made to determine whether epinephrine simulated a veratrine-like action upon the skeletal muscle of the frog. A weak veratrine action is characterized by a prolongation of the relaxation phase lasting longer then 0.3 second after stimulation of moderate intensity. Such a response may be initiated without veratrine by the use of very strong stimuli. None of the preparations employed demonstrated a veratrine-like action after injection of 2 cc. of 1:1000 solution of commercial preparations, or after the injection of a saline extract representing one adrenal of a 29 kgm. dog. Immersion of a normal frog muscle in epinephrine solution up to 0.01 per cent, demonstrated no veratrine-like effects.

A Study of Oil of Chenopodium and its Components. A. E. LIVINGSTON. From the Division of Pharmacology, Hygienic Laboratory, United States Public Health Service.

A study has been made in regard to the toxicity of various components of oil of chenopodium as separated by E. K. Nelson. Ascaridol, terpenes of oil of chenopodium, rearrangement product of ascaridol,

alpha glycol, and the oil itself have been used. In addition a study has been made of various samples of the oil obtained from different localities and prepared at different distilling plants.

In experiments on rabbits and earthworms, ascaridol is definitely shown to be the most toxic component of chenopodium oil. Ascaridol is more toxic than the oil itself, and samples of the oil having a high ascaridol content and high specific gravity are more toxic than samples of a low specific gravity and low ascaridol content. The experiments on earthworms were ordinarily made at room temperature (approximately 21°C.). If the temperature was increased to 37°C. the toxicity was greatly increased. Oil of chenopodium is more toxic for rabbits which have fasted forty-eight hours than for those receiving food regularly.

Certain Aspects of the Part Played by the Liver in the Regulation of the Blood Volume. PAUL D. LAMSON.

Further evidence was given in support of the view that the liver plays an important part in the fluid regulation of the blood. Curves were demonstrated showing the effect of the intravenous injection of 25 cc. of 0.8 per cent NaCl per kgm. during a period of ten minutes, on the hemoglobin concentration in the normal dog and after removal of the liver. Curves were also shown of the effects produced by the addition of epinephrine, histamine, and pituitrin, singly and in combination, both with and without the liver. The results were as follows.

The sodium chloride and water injection reduces the hemoglobin concentration to about 85 per cent of the original, this gradually returns to normal in between thirty and thirty-five minutes. When the liver is removed the injection of 20 per cent less salt solution causes a similar depression of the hemoglobin concentration but the curve does not return to normal for over two hours.

The addition of epinephrine to the salt solution caused a great increase in the rate at which the hemoglobin returned to normal but no change when the liver was removed.

Histamine caused a concentration of the hemoglobin of the blood when added to the saline injected. No concentration took place after removal of the liver but the return of the curve to normal took place more quickly than with the saline alone.

Pituitrin caused a very great increase in time taken for the curve to return to normal, even greater than after removal of the liver. Mixing epinephrine or histamine with pituitrin did not overcome the effect of pituitrin in prolonging the time of return of the hemoglobin concentration to normal.

If hemoglobin can be used as an index of the fluid content of the blood which must first be determined more definitely, these curves would indicate the fluid content of the blood in the condition produced. Epinephrine and histamine would increase the rate of fluid loss. In the case of epinephrine the increase in rate would take place almost exclusively in the liver. With histamine the increase in rate of fluid concentration would occur largely but not entirely in the liver.

Pituitrin on the other hand would decrease the rate of fluid concentration from the circulation very greatly, holding the fluid injected in the circulation for over three hours. It also overcomes the action of epinephrine and histamine in their increase in rate of fluid loss. The increased rate of fluid loss after epinephrine has been accounted for by Lamson by constriction of the hepatic veins and an increased filtration pressure in the liver due to obstruction of the flow.

The rapid rate of fluid concentration after histamine may be accounted for by a general increase in venous pressure. As there is an increased vena cava pressure in this condition the filtration is not localized in the liver as in the case of epinephrine. The mechanism of the pituitary action is being investigated. The observation has been made by the author that there is an enormous increase of portal pressure when epinephrine and pituitrin are mixed, yet there is no increased rate of fluid loss. This brings up therefore the question of changes in permeability of the vessel walls.

It must be emphasized that until the question of redistribution of red cells is ruled out, these changes in hemoglobin concentration cannot be taken as absolute proof of fluid loss. It must also be pointed out that the doses of substances used are very great, the object of these experiments being to determine the part played by the liver, rather than the action of the substance themselves, which is now being undertaken.

A Proposed Method of Chemically Assaying Active Principles of the Digitalis Group. ARTHUR KNUDSON (by invitation), AND MELVIN DRESBACH. From the Department of Physiology, Albany Medical College, Albany.

The method here proposed is simple and depends upon a sensitive color reaction which takes place between the active principles of digitalis and similar substances and picric acid, as pointed out by Baljet in 1918.[1] It was then shown that the bodies having a typical digitalis action gave an orange red color with a dilute alkaline picrate solution.

This reaction has been used in developing a colorimetric method for determining the physiological activity of digitalis preparations. The method consists of first decolorizing the tincture or other solutions of digitalis bodies with a lead acetate solution and then removing the excess lead acetate with a sodium phosphate solution. The decolorized solutions are then treated with an alkaline picrate solution and the characteristic color develops in twenty minutes. As a standard for comparison an Ouabain solution was prepared which gives a color reaction equal to that of a standard tincture assayed by the Hatcher cat method.

The method has been applied so far to tinctures, fluid extracts, infusions, purified preparations of digitalis and also to some of the active principles. The potency of these preparations as determined

[1] Baljet, Henry, Schweiz. Apoth. Ztg., 1918, lvi, 71-73 and 84-88.

by the above chemical method has been compared with that found by the bio-assay method of Hatcher and Brody.[2]

In the case of tinctures and infusions the variations from the bio-assay are in most cases less than 10 per cent. With the fluid extracts, purified preparations and the pure principles the agreement on the whole is not quite so close, but in the great majority of the specimens tested the difference is less than 20 per cent. This agreement between the chemical and the bio-assay method is well within the limits of accuracy of any biological assay.

The Action of Certain Drugs on Respiration. CARL F. SCHMIDT (by invitation), AND W. B. HARER (by invitation). From the Laboratory of Pharmacology of the University of Pennsylvania.

Using decerebrated cats, expiratory and inspiratory responses to CO_2 inhalation were estimated by recording intrathoracic pressure, by means of the mediastinal space and without opening the pleural cavity.

Morphine, heroin, and codeine depress or remove the expiratory response without interfering with the inspiratory, whenever the rate of respiration is slowed by small doses.

The smallest effective dose of morphine was 0.001 gram, the average being 0.005 gram. Larger doses frequently accelerate respiration, the expiratory response returns, and reflex excitability is increased, even to the point of convulsions.

Heroin is effective in doses of 0.125–0.25 mgm., larger doses producing progressive depression only.

With codeine, doses of 30–65 mgm. are required to remove the expiratory response, and 80–120 mgm. may cause tremors or convulsions.

This expiratory depression seems to be a cause of the slower rate, for very gentle suction, applied only during expiration, causing more rapid emptying of the lungs, results in an acceleration to the normal rate, while the Hering-Breuer reflexes are exaggerated showing that the vagus mechanism is not depressed. After section of the vagi this expiratory depression is elicited more readily than before, so that the action is apparently a central depression, partly antagonized by vagus impulses.

It is possible that this selective depression of expiration, by doses that need not produce narcosis, is back of the specific action of these drugs in cough and dyspnea.

Other depressant drugs also depress expiration more than inspiration, but only in narcotic doses, and only with simultaneous depression of inspiration. Ether and urethane usually produce an expiratory rhythm which persists up to the point of dangerous narcosis. With all the drugs tried—ether, chloroform, hydrated chloral, urethane, magnesium, and luminal—expiration is the first to go as narcosis is deepened, and the last to return on recovery.

[2] Hatcher, R. A., and Brody, J. G., Am. Jour. Pharm., 1910, lxxxii, 360-372.

The Mechanism of Cocaine Fever. H. G. BARBOUR AND M. D. MOISE (by invitation). From the Department of Pharmacology, McGill University.

In dogs given subcutaneous injections of 20–30 mgm. per kilo cocaine hydrochloride, the rise in body temperature is associated with an increase in the blood concentration. The percentage of blood solids and the red blood cell count both increase coincidentally with the temperature. For instance in one dog a rise of 0.8°C was accompanied by an increase in the solids from 17 per cent to 18.4 per cent, the red blood cells being increased from 6,000,000 to 8,300,000. The return of the temperature to normal was preceded and then accompanied by dilution of the blood.

This polycythemia indicates a general thickening of the blood for it is just as evident in the femoral artery as in the ear vein. The disturbance thus produced in the heat eliminating mechanism is of greater significance for the production of fever than the muscular activity.

Cocaine fever can be superimposed upon coli fever in dogs, the drug causing further increases both in blood solids and in temperature. Its production under anesthesia is less constant and likely to be delayed, but when it appears the blood concentration is also seen.

The Physiological Assay of Pituitary Extracts. ERWIN E. NELSON (by invitation). From the Department of Pharmacology, University of Michigan.

The results of the assays of ten commercial preparations of pituitary extract are presented. In spite of the fact that most of these preparations are advertised as having been "physiologically standardized," some of them are ten times the strength of others. It is imperative, therefore, that there be incorporated in the next United States Pharmacopoeia a method of standardization that will insure a reasonable uniformity of strength. Criticisms of the two methods now in use are given. The pressor method of assay is satisfactory for the comparison of a large number of preparations, because of its ease of technic, simplicity of interpretation, and relative certainty of results. In this laboratory the technic is modified by using morphine with chloretone as the anaesthetic, and by cutting the vagi. The method is not believed to be desirable as official for reasons given: (1) It is illogical to use the pressor activity of a drug as the measure of its oxytocic strength; (2) there is some considerable evidence that the pressor and oxytocic principles are not the same; (3) the results of assays by the two methods are not always parallel; (4) the pressor method requires the use of a large number of dogs, which are not always available, and to the use of which there is considerable opposition in some quarters. Rabbits are not satisfactory for this test.

The oxytocic test is much more difficult of satisfactory execution than the pressor test. It has been found that by using the uteri from virgin guinea-pigs, weighing from 175 to 250 grams, the occurrence of un-

satisfactory preparations can be reduced to a minimum. The uteri must be handled very carefully and weighted very lightly. The use of histamine as a standard, as recommended by the United States Pharmacopoeia IX is found unsatisfactory for these reasons: (1) it is a complex organic substance, apparently not stable, and in our experience the solutions lose in activity when sterilized by boiling; (2) it is active in extremely high dilutions, thus emphasizing errors of dilution in preparation; (3) it is scarce and expensive. The use of potassium chloride as a standard, as recommended by Spaeth has proved unsatisfactory, because of the fact that the irritability of uteri toward pituitary extract and potassium chloride does not vary in a parallel manner. The irritability to potassium chloride increases in the course of an experiment only to a slight degree and then rapidly falls, at a time when the irritability to pituitary extract is still rising. In spite of the difficulties of the oxytocic method, it is considered preferable for the official method, because it is the logical method for assaying a drug used chiefly for its oxytocic action, and because it reveals differences of strength not always shown by the pressor method.

The use of any of the so-called "standard extracts" is opposed on theoretical grounds, as failing to make possible an absolute evaluation. It may however become necessary to employ them because of the lack of a simple substance giving a reaction qualitatively similar to that given by pituitary extract.

The Action of Pilocarpine Upon Sympathetic Nerve Endings. C. W. EDMUNDS, University of Michigan.

Notes on the Action of Atropine. V. E. HENDERSON.

1. An attempt was made to estimate the amount of atropine necessary to depress completely the various nerve organ complexes in the body affected by the drug. The order appears to be heart, salivary glands, intestinal muscle, bladder muscle.

2. The stimulation of urinary bladder nerve muscle complex by pilocarpine is abolished by atropine, but even large doses of atropine do not diminish the contractility of bladder to nerve stimuli.

Quantitative Studies of the Colloidal Properties of Arsphenamine and Allied Products. G. W. RAIZISS (by invitation). Various investigators have shown that the cause of the untoward reactions following intravenous arsphenamine injections cannot be attributed to any considerable extent to 3-amino-4-hydroxyphenylarsenious oxide, "arsenoxide." Furthermore, no other chemical impurity or impurities in the drug, designated by Schamberg, Kolmer, and Raiziss as substance X, have as yet been isolated or identified. A study of the colloidal properties of arsphenamine suggested itself with the hope that it may eventually aid in accounting for the above "reactions."

Previous workers have shown that this arsenical is of a colloidal nature. A study of its rate of diffusion as well as those of other organic

arsenicals used in therapy through parchment membrane was made. The following compounds arrange themselves thus: (1) arsphenamine; (2) neoarsphenamine; (3) silver sodium arsphenamine; (4) disodium (alkalinized) arsphenamine; (5) gold sodium arsphenamine; (6) disodium 3-amino-4-hydroxyphenylarsonate in which arsphenamine diffuses least and disodium 3-amino-4-hydroxyphenylarsonate most rapidly.

Arsphenamine in water medium diffuses twenty-five times as slow as sodium chloride; dissolved in methyl alcohol, it diffuses more rapidly. In water medium, its chlorine ions diffuse much more rapidly than the arsenical. In the dialysis of the alkalinized solution, the sodium ions also diffuse more rapidly than the arsenical. In neoarsphenamine, its sulfur diffuses at a more rapid rate than arsenic, possibly indicating a cleavage of the sodium formaldehyde sulfoxylate. The results with silver and gold sodium arsphenamines throw new light upon their compositions. According to Bauer, the silver compound is a homogeneous substance, while according to our results, no silver was found in the dialysate after thirty-six hours in one experiment and eighty-four hours in another despite the fact that the arsenic diffused more rapidly than in the case of arsphenamine. Similar results were obtained with the gold compound. This tends to prove that these compounds are mixtures of sodium arsphenamine and a colloidal metal rather than a homogeneous chemical compound.

The valence of the arsenic appears to be an important factor with regard to both therapeutic and colloidal properties, since the trivalent arsenicals are both more therapeutically active and more colloidal in nature than the pentavalent arsenicals.

Penetration of Kations Into Living Cells. M. M. BROOKS (by invitation). Division of Pharmacology, Hygienic Laboratory.

Nitella, a single coenocytic cell, several inches in length was placed in solutions (hypotonic) of Sr, Li and Cs dissolved in distilled water or balanced solution, for varying periods of time. The cell contents were then tested spectroscopically for traces of the salts used. These tests showed that the protoplasm is normally permeable to these salts and that penetration is more rapid in an unbalanced than in a balanced solution. This is the most satisfactory direct evidence of penetration so far demonstrated.

FUNCTIONAL EVIDENCE OF THE PHYLOGENY OF THE NERVOUS SYSTEM AS SHOWN BY THE BEHAVIOR AND RESISTANCE OF THE DEVELOPING RAT TO STRYCHNINE[1]

ERICH W. SCHWARTZE

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It was observed in the course of study of the toxicity of strychnine to brown rats, Rattus norvegicus, that certain peculiar reactions occasionally occurred in some of the smallest individuals. Since it was impossible to obtain young wild rats in sufficient numbers and of suitable and known ages, the investigation of these phenomena was undertaken upon immature white rats of the same species. This appeared to be a legitimate procedure, since it has been shown that the adult subcutaneous minimum lethal dose of strychnine sulphate was practically identical[2] for these two strains, being about 3 mgm. per kilo. Since it will also be shown that several infant brown rats behaved quite similarly to the albino rats, there is no reason to assume that the conditions found in the domesticated strain do not also exist for the other.

LITERATURE

Experiments dealing with the effect of age upon the reaction to and toxicity of strychnine have not been very numerous. No place of importance is given to this phase in chapters dealing with the subject of strychnine in any of the common reference texts, it being dismissed with a few general statements. The

[1] Preliminary report in Jour. of Pharmacol. and Exp. Therap. 1920, x, 235.

[2] Unpublished manuscript to be issued as Bulletin of United States Department of Agriculture.

most significant work seems to have been done by Falck, who, although presumably originally interested chiefly in his sensitive biological test for strychnine on mice (1), nevertheless made some very important communications upon this subject. He found (2) that for the mouse of seven days, the subcutaneous lethal dose of strychnine nitrate is about 1.9 mgm. per kilo; for one of ten days, 1.3 mgm.; for one of fifteen days, 0.75 mgm.; and for one of twenty days, 0.42 mgm. Thereafter the resistance gradually increases. For the mouse one hundred and fifty days old, the lethal dose is about 0.78 mgm. per kilo. Although some other investigators have not found such a low adult lethal dose as Falck, the underlying conditions may be taken as correct; namely that the mouse is born with what is herein designated as a relatively high natal resistance to strychnine, which declines up to a certain age, and that after this decline, a post natal resistance is acquired. Falck's data also show that during the period of decline of the natal resistance, there is a rather wide latitude between the minimum convulsive dose and the minimum lethal dose, and that during this stage the mice react with numerous spasms. During the development of the post natal resistance a sub-lethal dose produces only tremors. No convulsions are produced until the lethal dose is reached, such convulsions being single.

Falck (2) found that in guinea-pigs at birth the minimum convulsive dose is about 1.4 mgm. strychnine nitrate per kilo. The minimum lethal dose is about 1.87 mgm. The resistance of the guinea-pig gradually increases up to one hundred days of age, at which time the lethal dose is about 3.4 mgm. per kilo, and the convulsive dose 2.8 mgm. per kilogram. The guinea-pig usually reacts with numerous spasms to the convulsive dose. It is evident from his data that soon after birth the guinea-pig acquires a post natal resistance and that no decline in natal immunity is observed. That the absence of the relative natal resistance may be apparent rather than real is suggested by the fact that the guinea-pig is born in a relatively mature state, capable of performing complex movements. The period of decline might, therefore, have been eclipsed by the latter part of the period in utero.

Falck (3) found that the subcutaneous minimum lethal dose of strychnine nitrate for new born rabbits is about 7.0 mgm. per kilo, and the convulsive dose about 0.42 mgm. This relatively great resistance to strychnine declines up to the fortieth day when it practically completes its rapid descent. The minimum lethal dose at this time is 0.6 mgm. per kilo. At the eightieth and two hundred and fortieth day the minimum lethal dose is only very slightly less, being 0.57 mgm. per kilo. On the other hand, the convulsive dose reaches its lowest level on the tenth day, when it is 0.21 mgm., and gradually rises thereafter to a value of about 0.4 mgm. per kilo for the sixtieth to the two hundred and fortieth day. It is quite evident that the rabbit is born with a relatively high resistance to strychnine which it eventually loses and which it never again acquires. This is in contrast to the mouse and the guinea-pig. After the fortieth day there appear to be fewer spasms and a much shorter experimental period preceding death.

Behrend Lau (4) determined only the subcutaneous convulsive dose of strychnine nitrate in 0.2 per cent solution for young cats and dogs. The minimum convulsive dose decreased as the animal grew older. Several new born cats and dogs received 0.5 mgm. or more per kilo, which is relatively enough strychnine to kill an adult animal. Multiple spasms frequently occurred. Paul Bert (5) found that young cats and dogs were very resistant to strychnine. One eight to ten day old pup withstood a dose of 3 mgm. per kilo for five hours, at the end of which time it was killed. Another survived a dose of 12.3 mgm. Both had typical frequent convulsions. Another pup succumbed to 15 mgm. per kilo, given in two doses. The second dose of 11 mgm. was administered one hour and one-half after the first. A kitten five or six days old received 44 mgm. per kilo, and succumbed only after seventeen minutes. Hatcher and Eggleston (6) have shown the minimum adult lethal dose of strychnine sulphate for the cat to be about 0.32 mgm. per kilo and for the dog about 20 per cent more. These data of Lau and of Bert for the young, together with that of Hatcher and Eggleston for the adult dog and cat point to the conclusion that these animals

are somewhat tolerant at birth, that they lose this resistance, and develop no significant degree of post natal immunity to strychnine.

The maximum natal tolerance for the cat and dog, however, yet remains to be ascertained. The comparative frequency of spasms in the young and old animals also is wanting an accurate investigation. The influence of environment, stimulation, size of dose and the rapidity of its absorption, as well as a consistent use of terms designating the symptoms are of prime importance (20). Such type of an investigation would settle the matter of the reality of a range between the convulsive and the lethal doses for any species. By comparing Hatcher's and Eggleston's (6) data with that of Lau (5) it seems that a slight range might exist for adult cats and dogs.

All the data in the literature indicate that any statements as to the effect of age upon the toxicity of strychnine for young animals should be properly qualified. It seems necessary, particularly in describing sensitiveness in young animals, to differentiate between the convulsive and the lethal doses, since increased or decreased sensitivity may or may not exist, according to the standard selected for comparison. Tentatively, the animals thus far studied would seem classifiable into two groups, those in which no post natal immunity develops and those in which it does develop. The occurrence of a relatively high natal immunity for new-born animals seems to be a common characteristic, having been demonstrated in all the species studied except the guinea-pig. Its existence in this animal is probably only masked.

No attempt was made to review all of the literature on the subject of strychnine poisoning in man. There exists, however, the impression that children are less sensitive than adults (7). Recently, Cutler and Alton (8) described an interesting case of strychnine poisoning in a female child one year and two weeks of age. They state:

Admitted to the Massachusetts General Hospital, Accident Ward, at 5.30 p.m., November 25, 1915. One hour before admission the child had swallowed 12 to 14 pills which its father was taking for stomach

trouble.   The prescription showed these to contain $\frac{1}{30}$ grain of arsenic, $\frac{1}{60}$ grain of strychnine and 1 grain of iron.   The mother gave the following history.   Fifteen minutes previously the child stopped playing and had a slight general convulsion.   It vomited once shortly after.   The convulsive seizures were repeated in rapid sequence and the intensity increased.   By the time the hospital was reached, the child was cyanotic and in an almost continuous convulsion, in which opisthotonous was frequent, with periods of total respiratory inhibition.   Ether was at once administered. . . . .   Twice the anesthesia was removed, but the child on both occasions had severe general convulsive seizures with opisthotonous and respiratory inhibition and was saved only by using artificial respiration to get it under the anesthetic.

This is an instructive case, since the long interval over which spasms occurred, and their multiplicity are not typical of the usual picture of strychnine poisoning in the adult.   The observers themselves remarked about its similarity to clinical tetanus, a disease which is known to be usually characterized by numerous convulsions of longer duration than is observed in strychnine intoxication.   The system complex described in this case would seem to warrant the statement that it resembled that observed in very young animals.

METHOD OF EXPERIMENTATION

Strychnine sulphate, U. S. P., found by chemical tests to be free from brucine,[3] was used in all experiments.   The lower concentrations employed were made from a freshly prepared solution of 0.1 per cent strychnine sulphate in 0.9 per cent NaCl. The injections were made by means of tuberculin syringes into the subcutaneous tissue of the ventral abdomen wall.   In case of the very small rats the needle was inserted in the thigh, as this circuitous route helped to prevent leakage.   The dosage was always administered on the basis of body weight.   All rats were placed in small wire bottomed cages which offered them an opportunity for grasping.   The importance of this seemed to be considerable, since it was preferred by the rats and since instances

[3] Tested by Mr. H. E. Buc, formerly of the Bureau of Chemistry.

of premature and non-fatal spasms were apparently lessened. A more constant minimum lethal dose was thereby obtained. When necessary, the smaller rats were protected from cold by suitable application of heat.

CLASSIFICATION OF ANIMALS

Falck's data, which have already been discussed, were determined by this investigator from the point of view of the age of the animal. Although the functional development of the central nervous system apparently seems to follow more or less closely the age, in this study it was not found to be an infallible guide to the degree of the former. The results herein reported have therefore been regarded from the point of view of the functional development as well as of the age. The rats have been divided into the following groups:

1. The infantile stage. The rats in this stage have their eyes unopened or just beginning to open. There may be relatively little hair, or the coat may have just developed. The rats usually are unable to perform walking movements and are sometimes referred to as in the "crawling stage." The maximum age for this group is usually fifteen to seventeen days. It is conceivable, however, that this age limit may vary in different strains and under different conditions of growth, etc.

2. The weaning age. This state succeeds the first one from which the rat gradually passes. There is no hard and fast definition, the change being a gradual one over the course of a few days. The duration of this state would seem to be at least a week or ten days, and is at an end by about the twenty-fifth day of age. At this time the rat should be capable of leading an independent existence. It has acquired a good coat of hair, its eyes have been opened for some time, and it is agile in its movements.

3. The growth stage. This succeeds the second, and continues until adolescence. Early in this stage weight may be taken as an additional criterion.

4. The mature or adult stage. Although the mature rat may continue to put on weight for a long time this stage begins about the time of sexual maturity.

EXPERIMENTAL RESULTS

In table 1 there are given data showing the effect of dilution upon the toxicity of the strychnine sulphate administered sub-cutaneously to ten rats from the same litter. Although it was theoretically desirable to administer amounts of fluid approximately equal to that injected into adult rats, it was impracticable. Leakage of serum or injected fluid occurred at the site of injection, especially during the spasm. Too great dilution decreased

TABLE 1

The effect or influence of the dilution of strychnine sulphate solution upon its toxicity (litter "S", eighteen days old).

WEIGHT OF RAT	STRYCH- NINE SUL- PHATE	STRYCHNINE SUL- PHATE INJECTED		REMARKS
		Strength of solution	Amount	
grams	mg. per kilo	per cent	cc.	
22	0.5	0.02	0.055	Died in 4½ minutes, 2 spasms
19	0.5	0.02	0.05	Died in 5 minutes, 2 spasms
19	0.5	0.02	0.05	Died in 5½ minutes, 1 spasm
20	0.5	0.005	0.20	Many spasms. Died in 25 minutes
21	0.5	0.005	0.21	Many spasms. Lived
20	0.5	0.005	0.20	Many spasms. Lived. Was taken once for dead
21	0.6	0.005	0.25	Died in 4½ minutes 1 spasm
21	0.6	0.005	0.25	Died in 8 minutes 1 spasm
21	0.6	0.005	0.25	Died in 8 minutes 2 spasms
21	0.6	0.005	0.25	Died in 8 minutes 2 spasms
23	1.0	0.005	0.46	Died in 3 minutes 1 spasm

the toxicity of the drug. It was found in this series that 0.005 per cent strychnine sulphate solution is at least 20 per cent less toxic than 0.02 per cent solution. The difference in toxicity is evident from the length of the pre-spasm period as well as from the duration of and the behavior during the experimental period, and presumably is due to the different rates of absorption of the drug. It illustrates, nevertheless, one of the difficulties to be encountered in the determination of lethal doses, and in the proof

TABLE 2

Toxicity of strychnine sulphate for infant rats (in the "crawling stage")

LITTER NUMBER	AGE	WEIGHT	NUMBER OF RATS	STRYCHNINE SULPHATE INJECTED		REMARKS
				Amount per kilo.	Strength of solution	
	days	grams		mgm.	per cent	
A	10	12.0	3	8.0	0.04	Died in 35 to 50 minutes. Almost continuous extensor spasm. Lividity marked
G	6	6.5	3	8.0	0.02	Died in about 80 minutes. Many tonic convulsions
A	10	12.0	3	4.0	0.01	Died in 75 to 100 minutes. Many tonic and clonic spasms
A	10	12.5	3	2.0	0.01	All lived. Many clonic spasms, begun in 4 to 5 minutes, over in 2½ hours
BC	12 to 14	19.0 to 21.0	6	2.0	0.02	4 lived, 1 died, 1 killed by mother and had spasm while eviscerated
F	15	12.0	1	2.0	0.005	Died in 8½ minutes. Had two tonic spasms
F	15	11.0	2	1.5	0.005	Extensor spasms. Returned to mother in 30 minutes in paralyzed state. Dead next morning
D	15	15.0	1	1.5	0.02	Died in 34 minutes
D	15	15.0 to 16.0	4	1.0	0.02	2 died in 15 to 60 minutes, had a few spasms; 2 had many spasms and recovered
E	15 to 17	20.0 to 23.0	2	1.0	0.02	All lived, many spasms, more clonic towards the last
H (wild)	?	24.0 to 27.0	2	1.0	0.005	Many spasms. Died in 15 to 20 minutes. Eyes open for first time on day of experiment. Rather active and agile
E	15 to 17	23.0 to 24.0	2	0.75	0.02	Both lived, many spasms
F	15	12.0	2	0.75	0.005	Died sometime after 30 minutes. Clonic spasms toward the last. Considerable paralysis
E	15 to 17	23.0	2	0.6	0.02	Lived, many spasms
E	15 to 17	23.0 to 26.0	2	0.5	0.02	Lived, many spasms
H (wild)	?	23.0 to 28.0	2	0.5	0.005	Lived, no spasms

Litter D. Eyes were unopened; hair was fairly thick, possessed considerable agility. Peculiar in that paralytic action of strychnine marked.

Litter E. Only three eyes in entire litter were opened. 4 more eyes opened the next day. Seemed to be a little stunted.

Litter F. Eyes of survivals open the next day. Paralytic action was especially marked in three higher doses. Clonic movement was more evident toward the last.

Litter H. Wild rats. Age unknown. Eyes open on day of experiment for first time. Rats rather active and agile.

of the existence of a true convulsive sub-lethal dose.   Hatcher and Eggleston (16) found that 0.0128 per cent strychnine sulphate solution administered subcutaneously is about 20 per cent less toxic than 0.128 per cent solution.   Such diminution in potency of the drug can be neglected since it tends to lessen rather than to magnify the contrasts which are shown in this paper.

Table 2 shows the results of experiments on infant rats. As judged by the weight and age, these rats were in various stages of physical and nervous development, but still were very immature.   The data have been presented to permit of comparison of groups from individual litters as well as each individual. From the results it is evident that certain of those ten, twelve and even fourteen days of age survived a dose of 2 mgm. of strychnine sulphate per kilo, while for certain of the other rats the lethal dose was decidedly less.   The contrast is not as marked as will be shown (table 3).   There is positive evidence, nevertheless, that the infant rat declines in its resistance some time after birth.

The gradual loss by these infant rats of their natal resistance to strychnine is only one of the important findings.   The symptoms of strychnine intoxication also differed in many respects from those of the adult.   The initial convulsion occurred rather soon after the injection.   Practically all the rats enumerated in table 2 had many spasms, although some received more than the lethal dose of strychnine.   Usually, the convulsions were not unduly long and possessed a distinct clonic tendency, particularly if sublethal doses had been administered.   The occurrence of spasms ceased any time from about thirty minutes to three hours, apparently depending upon the dose, the dilution of the drug and the sensitivity of the animal.   The ability for recovery of respiration was well illustrated by the fact that several animals were discarded as dead and which several hours later were found to be alive.   Some time after a severe spasm had passed off the apnoea could be interrupted by appropriate stimuli, which if severe enough would throw the animal into another convulsion. Although earlier in the life of the rat a wider latitude between the minimum convulsive and lethal doses than here demonstrated

probably exists, the latitude here shown is sufficient to leave no doubt as to this also being characteristic of the infant rat. The size of the largest doses survived was a little less than that withstood by adult rats, but it is necessary to qualify this by stating that the very youngest rats were not tested. The chief importance is attached to the demonstration of the decline rather than to those absolute amounts which represent the extremes of tolerance.

The character of the spasms depended to a certain extent on the method of treatment as well as upon the size of the dose. The rats were kept in wire bottomed cages, and, presumably as a result of an attempt at grasping, the first spasm was chiefly tonic. Thereafter, the rats had usually lost their power to maintain an upright position and the subsequent spasms sometimes appeared more clonic. Placing a rat in the upright position tended to convert the clonic into a tonic convulsion. Shaking a rat in a clonic spasm had the same effect. In the rats which received the larger doses, particularly those receiving 8.0 mg. per kilo. the spasms were distinctly tonic and of long duration.

The rat at this stage presents similarities to the lower vertebrate. Frogs kept at 30°C. in contrast to the usual picture described of those kept at lower temperatures have multiple spasms of relatively short duration separated by intervals of relaxation (9). There exists also an appreciable range in frogs between the lethal and the convulsive doses. This qualitative similarity between the infant rat and the frog is interpreted to mean that at a certain time early in its life the rat gives unmistakable functional evidence of the evolution or primitiveness of its central nervous system.

Table 3 gives the results from the injection of strychnine into young rats which were approaching the time of weaning. By the end of this stage, they had acquired considerable ability to walk and to run, and were almost ready to begin an independent existence. The lethal dose, generally speaking, was 0.5 mgm. per kilo. The convulsions tended to be tonic and usually appeared within several minutes of the injection. Respiration was usually not reinstated after the first or the second spasm.

Rats in this stage, therefore, are more sensitive to strychnine and die from smaller amounts and sooner than rats in the infantile stage. They have taken on or are beginning to acquire the adult type of reaction in that they usually die very soon with the occurrence of but one or two spasms.

TABLE 3

Toxicity of strychnine sulphate for young rats approaching the weaning age

LITTER NUMBER	AGE	WEIGHT	NUMBER OF RATS	STRYCHNINE SULPHATE INJECTED		NUMBER DIED		NUMBER LIVED	
				Amount per kilo	Strength of solution	Having one spasm	Having several spasms	Having no spasm	Having several spasms
	days	grams		mgm.	per cent				
X	24	17 to 23	9	0.125 to 0.5	0.005	0	0	9	0
T and U	24	17	1	0.5	0.02	0	1*	0	0
W	23	20	3	0.5	0.02	0	3*	0	0
S	18	19 to 22	3	0.5	0.02	1	2*	0	0
R	18	19 to 20	2	0.5	0.02	0	2	0	0
S	18	20 to 21	3	0.5	0.005	0	1†	0	2†
V	21	20 to 22	2	0.5	0.02	0	0	2	0
S	18	21	4	0.6	0.005	2	2*	0	0
V	21	21 to 22	4	0.75	0.02	2	1*	0	1†
R	18	20	3	0.75	0.02	2	1*	0	0
S	18	23	1	1.0	0.005	1	0	0	0
V	21	20 to 21	2	1.0	0.02	2	0	0	0
W	23	22 to 24	3	1.0	0.02	3	0	0	0
T and U	24	20 to 21	2	1.0	0.02	2	0	0	0
R	19	19	1	1.0	0.02	1	0	0	0

* Two spasms.
† Many spasms.

A litter of 5 wild rats, each weighing 22 to 26 grams, received doses of strychnine varying from 0.5 to 3 mgm. per kilo. The 3 mgm. dose proved to be fatal. These rats were very wild, active and agile and ate readily of the general rat diet. The behavior of these rats indicates, however, that they must have been considerably older than their weight would signify. Other experiments (table 2) show, however, that the wild rat is just as sensitive and behaves similarly to the white rat.

Table 4 shows the results from the injection of strychnine into weaned rats which had not yet reached the stage of maturity. It is evident from these data that these rats were somewhat more resistant than young rats in the weaning stage, but less so than adult animals.[4] It is also apparent that the dilution of

TABLE 4

Toxicity of strychnine sulphate for weaned growing rats approaching the stage of maturity. Solution injected subcutaneously on the abdomen

NUMBER OF RATS	WEIGHT	STRYCHNINE SULPHATE INJECTED		FATALITIES	REMARKS
		Amount per kilo	Strength of solution		
	grams	*mgm.*	*per cent*	*per cent*	
1	36	0.5	0.02	0	35 days old
2	36	0.75	0.02	50.0	35 days old
2	40	1.00	0.02	0	35 days old
2	98–102	1.00	0.01	0	
2	35	1.5	0.01	50.0	35 days old
3	108–111	1.5	0.01	0	
7	50–83	1.5	0.02	0	
1	92	1.5	0.10	100.0	
4	34–53	2.0	0.01	0	36 days old
5	32–42	2.0	0.02	80.0	35 days old
5	48–75	2.0	0.02	40.0	
4	84–100	2.0	0.033	0	
16	102–128	2.0	0.10	37.5	
2	150–168	2.0	0.10	0	
4	106–113	2.3	0.10	25.0	One more rat thrown into fatal spasms by handling
3	72–92	2.5	0.033	100.0	
1	125	2.5	0.033	0	
6	92–106	2.5	0.1	83.3	
4	83–90	3.0	0.033	100.0	

strychnine injected influenced the toxicity, although there are not enough data to state definitely how much difference this made in these cases. Since individual differences occurred at this stage, a very large number of experiments would be required

[4] The minimum fatal dose of strychnine sulphate administered subcutaneously to the adult rat was previously stated to be 3 mgm. per kilo in 0.1 per cent concentration.

to rule out this factor. The general trend of affairs, however, is demonstrated with certainty, namely, that as the rat matures it rather rapidly develops a tolerance to strychnine which may be termed an acquired post natal resistance.

It was stated that rats about to be weaned had taken on or were taking on an adult type of reaction to strychnine, in that there was a tendency for but one or two spasms and that the latter were more of a tonic nature. There were, however, some exceptions, and also a few peculiar reactions. In two of the rats noted in table 4 death failed to occur after numerous spasms which were distinctly clonic in type. In the case of two wild rats not enumerated in this table, incessant running motions occurred for over an hour, while the animals lay helpless on their sides. One of them recovered. The litter of four white rats to each of which 2.3 mgm. strychnine sulphate per kilo were given (table 4) seemed to be peculiar in that two of these rats had an initial tonic spasm, and became unable to stand. Later tonic spasms were confined mainly to the hind limbs, while the fore limbs executed running movements or clonic spasms. One of these died while the other survived eighteen hours, at which time the hind legs were completely paralyzed. The third rat of this set was thrown into an initial and fatal convulsion by handling, ninety minutes after the injection. The fourth had no spasms.

While no special experiments were performed to ascertain the nature of these exceptions, several causes are to be considered: (1) The functional metamorphosis of central nervous system, the type of which is usually predominantly shown by the end of the weaning stage, is occasionally very incomplete; (2) The latitude between the convulsive and the lethal doses, although ordinarily insignificant, was sufficiently conspicuous in a few animals to have been accidentally encountered; (3) The method of experimentation used may have tended to obliterate a very small latitude between these doses which may have been evident by other means of experimentation. (Whether the wire-bottom cages used in order to give the rat opportunity for grasping were always a real or a false security is remote from this

question, but it is true that given this opportunity rats accepted it. This may have affected these data slightly); (4) The difference in behavior of the fore and the hind limbs indicates that these pairs of appendages possess qualitative differences. This is also supported by observations on adult rats. While the hind limbs were paralyzed, but still capable of spastic contraction from suitable local stimuli, the front limbs were presumably under control of the motor cortex, since they were used in crawling.

DISCUSSION

Since data exist concerning the effect of development or age upon the toxicity of and the reaction to strychnine for all of the other common laboratory animals, these experiments upon the rat would seem to have a general as well as a special significance.

The rat and the mouse are the only animals thus far described to show both the phenomena described as natal and post-natal resistance to strychnine. The rat apparently exhibits the latter to a much more marked extent, and for this reason as well as for its more convenient size it would seem to possess experimental preference over the mouse. Although it seems not unlikely that the guinea pig also passes through a period of natal immunity *in utero*, its usefulness is abeyant, at least as an analogue of the rat and mouse, until this point shall have been proven. The cat, dog and rabbit apparently do not possess the phenomena of post-natal resistance, and are useful because of this fact, since they furnish a different experimental type. The significance of both these types lies, therefore, in our possession of quantitative and qualitative functional criteria for the study of the comparative pharmacology of strychnine.

Although since the advent of the modern conception of rational therapeutics strychnine has suffered considerably in therapeutic prestige, it has nevertheless gained an important rank as an experimental drug (10) (11). It would also seem from consideration of the evidence herein given, that its usefulness as a means to an end is again extended. With the exception of a few physiological observations, the evidence of phylogeny of the central nervous system has been collected chiefly from the morphological

standpoint. It appears in the light of the experiments herein reported together with the evidence in the literature that strychnine could be used here with profit. The analogy between the functioning of the nervous system of the frog (9) and of the infant rat is not nearly as remote as the species are distant. In the mammals so far studied and presumably in all, there must exist, at some time, some functional evidence of a recapitulation of some stage or stages of the prototype. Strychnine may be one of the means of bringing out some of this evidence.

The suggestion that strychnine be used for studying functionally the development of the influence of the more anteriorly situated centers upon the segmented reflex is a logical one. This drug predominately spends its effects upon the primitive and elemental units of the nervous sytem (the segmental reflex paths). The persistence with which strychnine has tended to maintain its site and purity of action particularly throughout the evolution of the higher mammals is shown by the fact that strychnine convulsions may be obtained immediately after spinal cord transection (12).[5] Since this operation in higher animals temporarily renders functionless for usual purposes the somatic reflex mechanism, it presumably shows that the strychnine action at least is not subordinated or rendered functionless, to the degree that the normal function is.[6] The strychnine mechanism is, however, not entirely out of relation to the motor cortex (10). This is shown by the production of typical strychnine phenomena upon cortical stimulation. It is also evidenced by the qualitative difference between the behavior of the anterior and posterior pairs of appendages (13). In some experiments, both upon the young and the mature rat, one of the first signs of return of

[5] The influence of spinal cord transaction on the amounts of strychnine required to produce convulsions does not seem to have been determined. Such a determination might throw light upon the existence of a normally active influence or inhibition restraining convulsions in the intact strychninized animal, as well as upon the definition of the so-called "convulsive dose."

[6] An excellent consideration of spinal shock is given by Pike (12). Since the submission of this article Prewitt (Amer. Jour. Physiol., 1921, lvii, 291) has presented evidence indicating a possible relation between spinal shock and surgical trauma.

voluntary control in the convulsed musculature was the opening of the jaws. However, this was not due to any insusceptibility of the local nervous mechanism to strychnine. The jaws were often forcibly closed, and after the more severe spasms paralyzed. There also has been observed voluntary motion of the jaws while the rest of the body was in mild convulsions. This behavior observed in the jaw harmonizes with that difference observed between the anterior and the posterior limbs, and points to the conclusion that the motor cortex exercises a greater controlling and opposing influence to strychnine on the more anteriorly situated segments.

Strychnine is not the only experimental means that shows differences in the character of reaction of the segmental reflex arc. Spinal shock is less severe in young animals than in older ones (12). "On high spinal transection or on decerebration the reflexes of the hind limbs return sooner than those of the fore limbs" (12). In decerebrate rigidity the hind limbs (of cats) are affected less. These facts contrast with strychnine in type, because with strychnine, we deal with the removal of and the return of subordination of the segmental reflex unit to the higher centers, whereas with surgical operations on the brain and cord we deal with the removal of the higher controlling centers or the return to spinal autonomy. It is therefore logical from the point of view of the functional unit that we should look to the use of strychnine for studying the degree of influence of the higher centers on the primitive unit and also the effect of age or development on the degree of subordination.

Other sources for probable corroborative experiments are available.[7] It seems singular, if not significant, that in those animals (cats, dogs and rabbits[8]) which do not develop a postnatal immunity the pyramidal tract lies in the lateral funiculus, whereas in those that do develop a post natal immunity it lies

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[7] Coincidences in man between the time of myelinization of the pyramidal tract and the time of change of the Babienski reflex from plantar flexion to dorsal flexion at times have been regarded as significant.

[8] According to Gidley, J. W. (14) the rabbit should be deleted from Rodentia and placed in an independent order. Whether or not mammalogists will also use finer neurological criteria in debating Gidley's contention, remains to be seen.

in the dorsal funiculus. Still other species (bats and hedgehogs) are available in which the pyramidal tract is even more primitive, lying in the ventral funiculus, and for which we have conflicting or incomplete data (14), (15), (16), (17) for strychnine. Morphological and psychological studies have been made by Watson(18). Although he came to a negative conclusion as regards a relation between myelinization and function, it may be that one or both of these methods could be used advantageously along with strychnine tests.

Morphological and chemical differences (21) (22) (23) have been sought by different investigators in various conditions of the central nervous system. Spontaneous or elicited symptoms are sometimes sought. It is entirely possible that strychnine tests would give very definite and much desired information about the conditions of the nervous system, such as are found in diseased or in stunted animals, providing enough were known about the normal.

The strychnine test primarily is a test on the elemental unit of the central nervous system and possesses the following characteristics: It is qualitative in that the reaction is of different types, the infantile (representing the prototype), the adult, and a transitional between these two. The latter is important in that the metamorphosis is shown to be not abrupt. The strychnine test is quantitative, in that a reaction is obtained at all ages, from fairly definite amounts of strychnine. To be sure, the mathematical terms representing these doses are not true ratios or indices of the degree of development, but since they are reproducible, they are definite or fixed and thus relatively quantitative. The reaction is forced, in that beyond a certain point the elicitation of symptoms is not prevented by active or passive voluntary restraint (20). The type of reaction is predetermined and not dependent upon past experience with strychnine. Although this test is not free from the admixture of alien influences, such as fear, and desire for self-preservation, it is primarily within the realm of the physiological, rather than the psychological.

CONCLUSIONS

1. Sometime after birth the rat begins to lose its natal resistance to strychnine. During this interval, however, it still possesses the lower vertebrate type of reaction, namely, the tendency to have numerous spasms which are separated by periods of relaxation and apnoea, even though more than the lethal dose has been administered.

2. Several days after the opening of the eyes, after the rat has emerged from the so-called "crawling stage," it has usually acquired the adult type of reaction, namely: the tendency for but one fatal spasm. At this time the lethal dose is at its lowest limit, or 0.5 mgm. per kilo.

3. Subsequently the rat acquires what may be termed a postnatal immunity, which increases up to the time of maturity, at which time it has an absolute value of 6 times that exhibited on emergence from the "crawling stage."

4. Sufficient has been here demonstrated in the rat as well as by other investigators in the other animals to make feasible the beginning of an interesting investigation of the factors underlying species tolerance to strychnine.

5. These facts are indicative that the developing central nervous system of a rat (and presumably of other mammals) pass through certain functional stages of development from the lower prototype to the higher forms which are just as definite and as important functional evidence of the phylogenetic development of the nervous system as morphological, physiological or psychological evidence.

6. The effect of development or disease upon the toxicity and reaction to strychnine would seem advantageous as a method for studying the functional metamorphosis and pathology of the central nervous system, particularly in conjunction with other methods such as the physiological, morphological and the psychological.

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PHARMACOLOGICAL STUDIES ON ACETONE

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Previous observations on the effect of acetone made by different workers are not quite in agreement as to its action. Some of the experiments reported indicated that its pharmacological properties were not very marked, being considerably weaker than those of ethyl alcohol. According to Albertoni (1) large quantities may be given without producing serious effects. One gram of acetone per kilo given by mouth to dogs failed to cause any symptoms. After a dose of 5 grams per kilo blood pressure fell 10 to 15 mm. of mercury, while the pulse was at the same time accelerated and respiration was regular. In tests made on man slight narcosis was the only symptom he observed after feeding doses of 15 to 20 grams of acetone. Different results were reported by later investigators who have shown that acetone possessed considerable activity. Albertoni and Pisenti (2) stated that the administration of sufficient amounts produced injuries to the renal epithelium and caused albuminuria. Cossmann (3) observed depression of the nervous system and paralysis in animals that received acetone. Albanese and Parabini (4), who studied the action of various ketones, have shown that acetone produced incoordination, increased cardiac activity, and later paralysis of the central nervous system. According to these observers the hypnotic effect noticed after acetone was similar to that produced by other aliphatic ketones. From the more recent observations of Rhamy (5) it appeared that acetone injected subcutaneously into guinea-pigs was found to be more toxic than methyl alcohol. It is evident, therefore, from the above summary that acetone is a poisonous substance. Nevertheless, some writers regard it as non-toxic. A statement to this

effect was made by Hewlett (6), who maintained that acetone was not very poisonous. He stated further, however, that it possessed pharmacological properties that lie between alcohol and chloroform. In view of its occurrence in a variety of pathological conditions, being present in increased quantities in the blood in diabetes, carbohydrate starvation, cyclic vomiting in children, hepatic degeneration produced by chloroform, phosphorus and other poisons, its behavior in the body is particularly interesting. It may be added also that in a number of industries many individuals are exposed daily and for years to the action of acetone.

The acetone used in the present investigation was of highest purity made from the bisulphite by the Eastman laboratories. During our preliminary observations a few tests were also made with a commercial preparation, for comparison. No difference in the effect of the two could be noticed, but the purest quality acetone was employed in all our subsequent experiments. Our studies include observations on the effect of acetone on the circulation and respiration in cats and dogs, experiments on the isolated heart of frogs and turtles, determination of the rate of absorption from the stomach and intestine, the length of time it remained in the circulation, and the channels of elimination. Observations were also made on the effects of acetone on the nervous system.

THE INFLUENCE OF ACETONE ON THE CIRCULATION AND RESPIRATION

As already stated cats and dogs were the subjects of these experiments. Those on cats were carried out under ether or paraldehyde anesthesia. The experiments on dogs were conducted under morphine-ether anesthesia at first, but later in the investigations paraldehyde was used instead. Blood pressure was recorded by means of a mercury manometer. Respiration was recorded by a Becker plethysmograph (7), or by a tambour connected with the interior of a rubber bag made of a short length of inner tube.

Experiments on cats. Intravenous injections of acetone produced considerable disturbance of the circulation and respira-

tion. Comparatively small doses given in concentrations of 25 and 50 per cent caused depression of the circulation. After the injection of 1 cc. 25 per cent acetone per kilo blood pressure fell about 15 per cent, the effect being greatly augmented by larger doses. Two cubic centimeters of 25 per cent acetone per kilo often caused a fall in blood pressure amounting to 40 per cent.
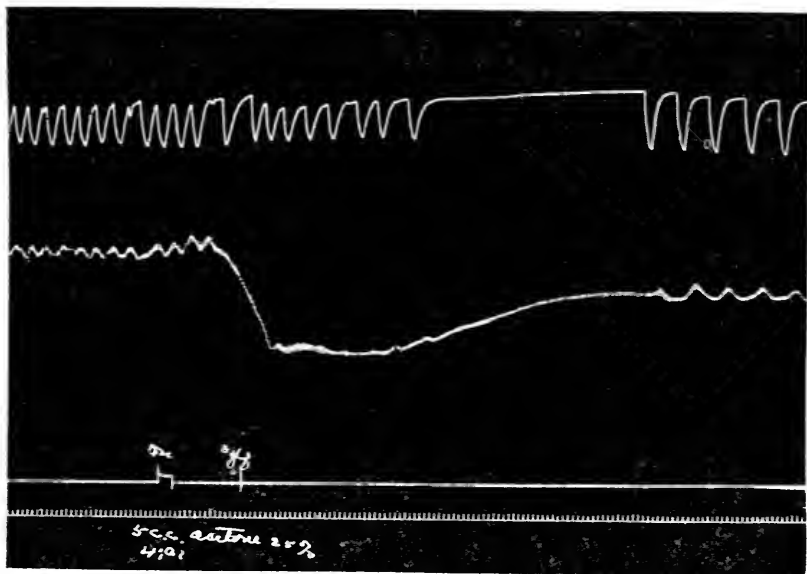
FIG. 1. EXPERIMENT 13. CAT, WEIGHT 2.4 KILOS

Ether anesthesia. Injected intravenously 2.08 cc. 25 per cent acetone per kilo. Blood pressure fell promptly and recovered more slowly. Respiration inhibited some time after blood pressure fell. Reduced one-half. Time in seconds.

Important factors influencing the action of acetone were the speed of injection and repetition of dose, the result in both cases being to greatly increase circulatory depression. Recovery was prompt in some cases, but in a number of experiments a tendency to prolonged depression was noticed. Tests with undiluted acetone indicated more profound disturbance of the circulation. Doses of 0.4 to 0.9 cc. per kilo lowered the blood pressure very considerably, often to about 20 mm. Hg. The effect of acetone

on respiration was in some respects different from that on the circulation. Although depression greatly predominated, not infrequently this was preceded by stimulation. Thus in one experiment frequency was increased from 23 to 50 per minute after the injection of 1 cc. per kilo of the 25 per cent solution. The depression observed varied in extent and duration. Decreased frequency with diminution of amplitude to complete suspension of respiration for periods of thirty to fifty seconds occurred in some experiments. Strong acetone caused paralysis of respiration after a dose of 0.4 cc. per kilo. The effect of acetone on the cat may be briefly summarized by stating that it depressed the circulation even when small doses were injected intravenously. Respiration may be stimulated for brief periods by small doses, while larger amounts always caused depression and paralysis which almost always occurred before heart action ceased. The heart sometimes continued to beat for several minutes after respiration was paralyzed.

Experiments on dogs. The changes produced in the circulation and respiration after the intravenous injections of acetone also varied in these animals with the size of the dose, the speed and the number of injections. Amounts of 0.5 to 1.0 cc. per kilo, whether given in a dilution of 25 or 50 per cent, produced a fall in blood pressure of 14 to 40 per cent, when the injection was rapid. In most experiments, however, the fall in blood pressure varied between 33 and 40 per cent, the cases in which the circulation was only moderately depressed being much smaller in number. In practically all experiments the effect set in very promptly when the injection was rapid, and was followed by recovery. The returning rise was slower, however than the descent of the blood pressure. The action on the circulation was different when the injection was less rapid. When the speed was decreased several times, the fall in blood pressure was about 7 to 10 per cent. Moreover, the effect was the same whether given in a concentration of 25 or 50 per cent. In several experiments, however, depression of the circulation was also considerable when acetone was injected slowly. This was often observed after a number of doses were given, those injected later being much more

effective.   Thus in one experiment 109 cc. of 25 per cent acetone
were given in eight injections to a dog weighing 5.7 kilos.   At first
a slight effect on the circulation was produced by the injection of
2 cc. per kilo of this solution, but an equal amount injected later,
with the same speed, lowered the blood pressure about 25 per
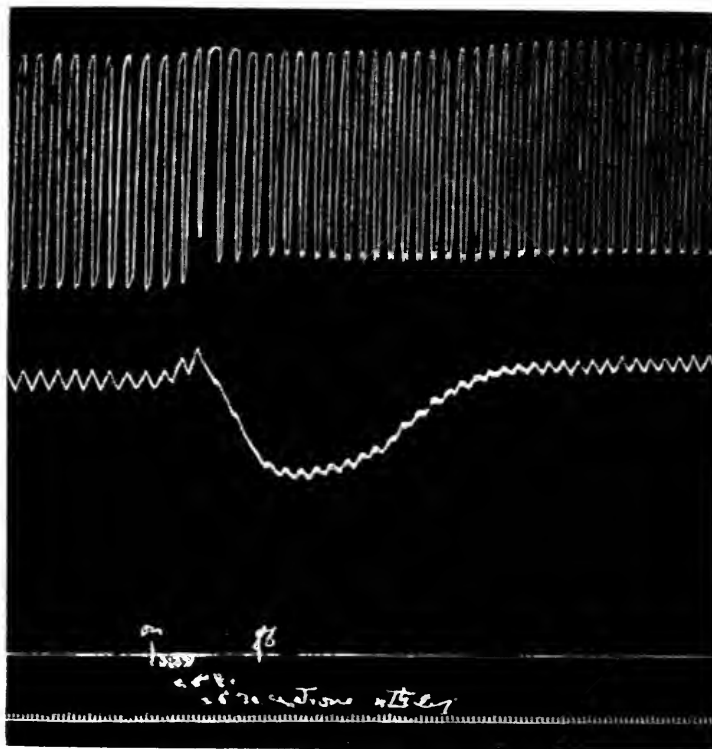
FIG. 2. EXPERIMENT 21.   DOG, WEIGHT 6.3 KILOS

Ether anesthesia, shows fall of blood pressure after rapid injection of 4 cc.
25 per cent acetone per kilo. Not much change in respiration. Compare with
figure 1. Reduced one-half. Time in seconds.

cent in one instance and 33 per cent in another.   The increase
in sensitiveness to acetone after a number of injections were given
was often very striking.   The circulation responded to small
doses by a very considerable and persistent fall in blood pressure,
whereas previous injections failed to produce any effect, though
the amounts given were much larger.

Acetone likewise caused disturbances of respiration, but the changes observed were different in some respects from those produced on the circulation. Doses of 0.5 to 1 cc. given in a concentration of 25 per cent, and in one experiment when 50 per cent acetone was used, stimulated respiration, if the injections were made with moderate speed. But the same amounts introduced more rapidly were followed by depression, decreased frequency being observed in some experiments, while in others respiration also because more superficial. After larger doses complete inhibition of respiration occurred which lasted a half to one minute. Other effects observed were irregularity of respiration and Cheyne-Stokes breathing which occurred in experiments even when no morphine was given. After several injections were made the respiratory center became more sensitive to acetone. Moreover, the effect on respiratory showed a tendency to persist, thus differing from the action on the circulation, which recovered soon after acetone had been given. Finally it may be observed that after lethal doses paralysis of respiration occurred some time before the heart stopped. The heart sometimes continued to beat three minutes or longer after respiration ceased.

Observations were also made on changes in the volume of the kidney after intravenous injections of acetone. The effects are shown in the following experiment. A dog weighing 13.8 kilos was given morphine-ether anesthesia, the vagi were cut, the abdomen opened, and one kidney enclosed in an oncometer, for the description of which the reader is referred to a previous publication by Salant and Livingston (8). Different amounts of acetone were injected. The volume of the kidney followed closely the changes in blood pressure, the effect being very striking when large doses were given, indicating that the depression of the circulation was of cardiac origin.

A number of tests were made on the reaction of the vagus mechanism after acetone. When the peripheral end of the cut vagus was stimulated by means of the faradic current arrest of the heart was elicited more readily after than before acetone, cardiac inhibition being very marked after considerable amounts

had been introduced. When the central end of one vagus was stimulated, the other being intact, cardiac inhibition was likewise greater after than before acetone. That inhibition was greater was also shown by examination of the pulse rate, when acetone was injected before or after the vagi were cut. The slowing produced by acetone was appreciably greater before than after section of these nerves.

THE INFLUENCE OF ACETONE ON THE ISOLATED HEART

The isolated heart of the frog and of the turtle was perfused with acetone in Ringer's solution, in concentrations of 1, 5 and 10 per cent, the method employed being essentially the same as that described by Salant and Livingston (9). Neither the frog heart nor the turtle heart reacted to 1 per cent acetone even when perfused for periods of three to eight minutes. But the effect was different with the stronger solutions. Five per cent acetone perfused four to five minutes greatly reduced the amplitude in the frog heart, and, to a much smaller extent, also the frequency of the contractions. In several experiments, however, the frog heart proved very resistant to the action of this concentraton of acetone, very little or even no depression being caused. Similar effects were observed when the period of perfusion was extended to fifteen or even to twenty-five minutes (fig. 3). The amplitude began to diminish as soon as, or shortly after, the solution of acetone came in contact with the heart. The rate of decrease was greater at first, but later it diminished more gradually until the force of the heart was practically uniform. The rhythm also showed some abnormality, a tendency toward group contractions often developing after the heart was perfused for several minutes. In all cases complete recovery took place when perfusion with acetone was followed by Ringer's solution alone.

In experiments with 10 per cent acetone cardiac depression was much greater (fig. 4), arrest of the heart following almost immediately after perfusion was begun. If the period of perfusion did not exceed two minutes, recovery gradually took place so that after two to three minutes perfusion with Ringer's solution heart action was the same as before the per-
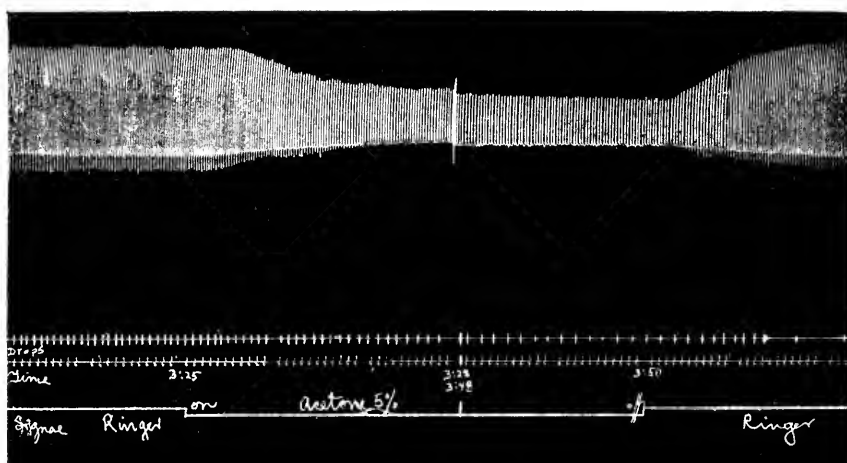
FIG. 3. EXPERIMENT 97. FROG HEART PERFUSED WITH 5 PER CENT ACETONE
IN RINGER'S SOLUTION FOR TWENTY-FIVE MINUTES

Amplitude was diminished rapidly at first then very slowly until no notice-
able decrease in force of heart beat could be observed. Notice tendency to
group contraction and prompt recovery of heart when Ringer's solution alone
was perfused after acetone. Reduced one-half. Time in five seconds.
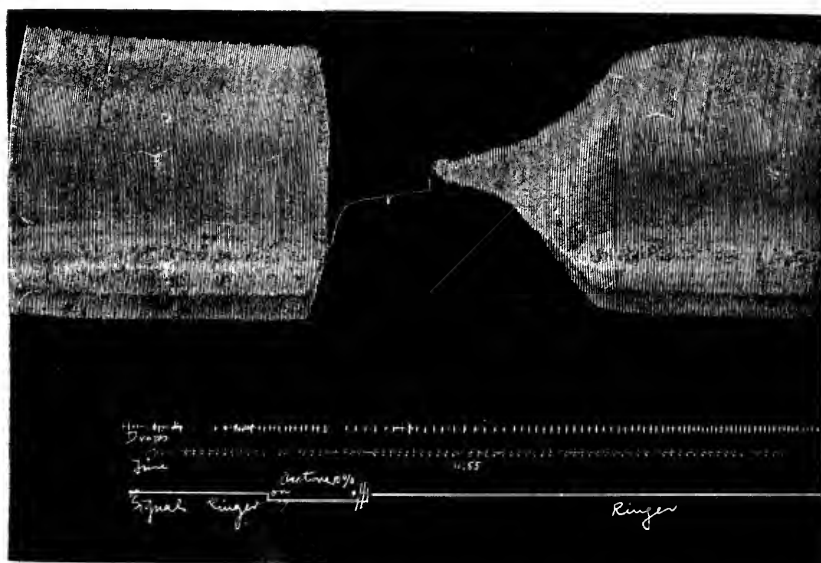
FIG. 4. EXPERIMENT 94. FROG HEART

Perfused for one minute with 10 per cent acetone in Ringer's solution caused
prompt arrest of heart, but heart action restored by Ringer's solution. Reduced
one-half. Time in five seconds.

fusion with acetone. After more prolonged exposure, seven to eight minutes, complete absence of cardiac activity ensued, and lasted several minutes. When acetone was discontinued recovery occurred even in these experiments. Furthermore, it is worthy of observation that substantially the same results were obtained when perfusion with acetone was repeated on the same heart a number of times, thus indicating absence of cumulation, or injury to the heart muscle.
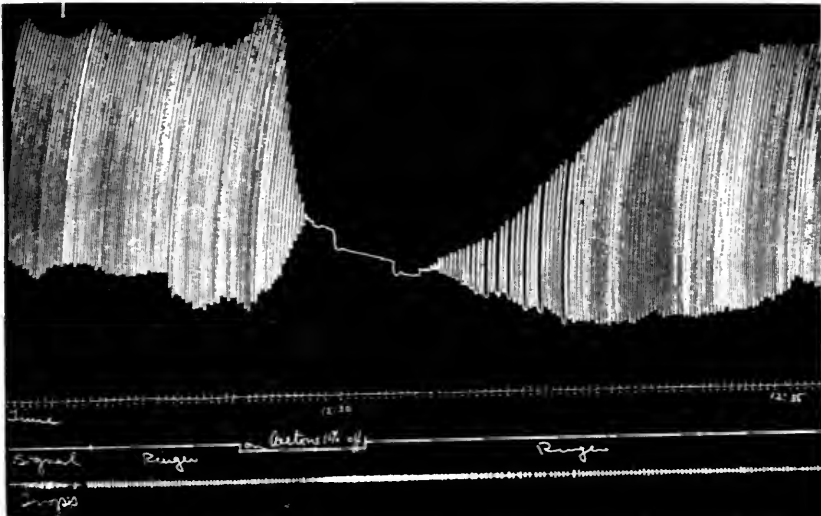
FIG. 5. EXPERIMENT 102. TURTLE HEART

Perfused with 10 per cent acetone in Ringer's solution for one minute. Heart action restored by Ringer's solution within five minutes. Time in five seconds.

Experiments on the turtle heart showed that the influence of acetone was different from that on the frog heart. The reaction of the turtle heart not only exhibited greater resistance to acetone than the frog heart, but qualitative differences could sometimes be observed. Arrest of cardiac action was often produced with a concentration of 10 per cent, but vigorous contraction could sometimes be seen, though the period of perfusion lasted for five to fifteen minutes. Moreover, while the force of the heart was diminished, its frequency was, on the contrary, increased.

Some differences between the heart of the frog and that of the turtle were also observed in their behavior after the perfusion with acetone was discontinued.   Recovery was more gradual in the turtle heart, although its frequency was greater than before the acetone.   In both cases, however, acetone affected the muscle substance directly, since its effect on the heart was the same before and after atropine treatment.

Experiments with acetone were also carried out on the heart of the turtle in situ.   Ringer's solution containing 10 per cent acetone applied to the heart failed to produce any effect, but stronger concentrations, 25 to 50 per cent, caused depression. The force and frequency were considerably reduced, and in some cases complete arrest of the ventricle occurred, the auricles, however, continuing for some time to execute feeble movements. Removal of the acetone after two and one half minutes resulted in considerable improvement, but it usually took several minutes before complete recovery occurred.

THE TOXICITY OF ACETONE

In the course of a large number of experiments in which acetone was given intravenously for the purpose of studying its action on the circulation and respiration narcotic effects were always observed.   After a few injections of acetone further administration of ether could be dispensed with.   It was found, however, that very large quantities were required to produce complete anesthesia.   Its effect was also tested in unanesthetized animals.   A dog weighing 7.3 kilos received by mouth, through a stomach tube, 14 cc. of 50 per cent acetone, but no symptoms were observed.   The same dog was given, two days later, 14 cc. of undiluted acetone by hypodermic injection.   Severe symptoms of intoxication developed immediately.   The gait became unsteady, and the dog was rolling about on the floor for some time, indicating cerebellar disturbance.   Within twenty minutes after the administration of acetone the dog vomited.   Twenty-five cc. acetone was given subcutaneously to another dog weighing 11.5 kilos and produced the following symptoms: incoördination, which was very marked, rolling movements, barking, and great excitement;

the dog also defecated twice shortly after the injection. It should be added, however, that dogs varied considerably in their resistance to acetone.

Experiments on frogs showed that these animals were very resistant to acetone. One cubic centimeter per 100 grams of frog injected into the dorsal lymph sac produced coma and paralysis within a few hours. Increased reflexes were also noticed, but this soon disappeared. The effect of acetone passed off during the night, and the next morning no ill effects of the treatment could be observed.

ABSORPTION AND ELIMINATION OF ACETONE

The speed of absorption of acetone when introduced into the stomach was studied in two experiments, in one of which 14 cc. of 25 per cent acetone was given to a dog weighing 7.3 kilos, in the other 20 cc. of 10 per cent acetone was given in this way to a cat weighing 1.5 kilos. In both cases the blood examined within 55 and 45 minutes respectively gave a weak but positive acetone reaction. Observations were also made on elimination and on the disappearance of acetone from the circulation, when given intravenously, subcutaneously or intraperitoneally. That acetone when present in the circulation in abnormal conditions is eliminated by the kidneys and lungs has been established by numerous observers. Its elimination by other channels, however, has not been studied. The only report we could find on the subject was a casual observation by Cossmann (3), who tested the vomitus in a case of poisoning resulting from the external application of acetone. According to this report acetone may be absorbed from the skin and excreted into the stomach. Unaware of this observation, we carried out the following experiments on two cats under urethane anesthesia. One female cat weighing 1.95 kilos was given 2 grams urethane intravenously, and one hour later it received 20 cc. of 10 per cent acetone intravenously. The cat was killed five and one half hours later. The contents removed from the stomach showed the ·presence of acetone. Another cat weighing 1.9 kilos received the same amount of urethane intravenously, and then 1 cc. acetone in 60 cc. normal

salt solution intravenously, the stomach having been previously ligated at both ends. The cat died during the night. Tests on the contents of the stomach showed the presence of large amounts of acetone, but none could be found in the contents of the intestine.

The length of time that acetone remained in the circulation was studied by intravenous injections. Tests made on the blood of one cat taken at frequent intervals showed that acetone was still present in considerable amounts eight hours after the injection. In another cat which received 4 cc. acetone subcutaneously the blood obtained twenty-four hours later gave a positive reaction for acetone. Tests made on the blood taken from dogs indicated that acetone disappeared faster from the circulation in these animals than in cats. The blood of treated dogs failed to show the presence of acetone twenty-four hours after its administration, and in one experiment no test for acetone could be obtained 4 hours after its subcutaneous administration, the amount injected in this case being 2 cc. per kilo.

Tests for acetone were made according to the Rothera method adapted by Wishart for blood and described by Joslin (10).

DISCUSSION AND CONCLUSIONS

When the results of the foregoing experiments are analyzed, it will be observed that acetone possesses a double action causing depression as well as stimulation, but it is chiefly a depressant. The evidence upon which this conclusion is based is briefly as follows. Even moderate amounts injected intravenously caused a very considerable fall in blood pressure, the diminution in the volume of the kidney which accompanied it showing that the depression of the circulation was cardiac in origin. Though respiration was stimulated by small doses of acetone, larger doses always caused depression both in cats and dogs. Speed of injection and repetition of dose were important factors in determining its action. Some difference, however, was observed in the reaction of these animals to acetone, as the effect was more marked and more prolonged in cats than in dogs, which may be accounted for by the different speeds of elimination in these animals. It

will be recalled that acetone was still present in the blood of cats 24 hours after its administration, but that it disappeared much sooner from the circulation in dogs. That acetone also depresses the central nervous system was shown by the narcosis and paralysis observed in experiments on frogs, cats, and dogs. The centers in the medulla, however, were variously affected, those of inhibition and vomiting being stimulated, while the respiratory center was stimulated by small and depressed by large doses. The influence on the isolated heart varied with the concentration, depression being much greater when it was perfused with 10 than with 5 per cent acetone, while a solution of 1 per cent failed to cause any effect even when the perfusion time was quite long. The change consisted in decreased force without noticeable alteration in the frequency of the heart, although in some cases slowing was observed. But in all experiments recovery occurred when acetone solution was followed by perfusion with Ringer's solution alone. It may be added that the effect on the heart of the turtle was less pronounced than that on the frog heart.

The results of our experiments would seem to contradict some of the statements in the literature concerning the action of acetone. We never observed cardiac acceleration such as reported by Albertoni (1) or Albanese and Parabini (4). Nor could we corroborate the statement of Albertoni that after large doses respiration was regular. As to the potency of acetone it is evident from our investigation that its action is not very strong, but is nevertheless considerable. It is particularly interesting to note that its effect was greatly augmented when a number of just active or subminimum doses were given. The increased sensitiveness thus shown may be of some significance in pathology. Though the amounts of acetone present in blood in acidosis are comparatively small, it should be borne in mind that the tissues and organs in the body may be exposed to its action for a considerable period of time. There is therefore no justification for the assumption by some writers that acetone is without effect in those conditions simply because its concentration in the blood is low.

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THE TOXICITY OF SKATOL

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The products of intestinal putrefaction have long been regarded by clinical observers as factors in the causation of intestinal autointoxication. Support for this view was also furnished by experiments on animals and by observations on the effects produced when these substances were fed to human beings.

According to Nencki (1) 2 grams of indol may produce diarrhea and hematuria. In an extensive series of observations made by Herter (2) indol administered to rabbits caused depression of the circulation and respiration, and clonic convulsions. When fed to the human subject it gave rise to symptoms of neurasthenia, causing fatigue, insomnia and other effects indicative of mental disturbance. Experiments have also been reported on the action of skatol. Brieger (3) fed 2 grams of the substance to a dog and gave 5 grams more the next day, but failed to observe any ill effects. Positive results, however, were obtained by Rovighi (4), who studied the action of indol and skatol in different animals and reported that both of these substances produced symptoms of cerebral depression, feeble heart action, renal injury, constitutional disturbances and retention of feces. When given subcutaneously to rabbits, doses of 1.5 to 2 grams per kilo caused death within forty-eight hours. The interest which attaches to skatol as a factor in the production of toxemia prompted us to make additional observations on its behavior in the body. We carried out, therefore, experiments on different animals. Frogs were used for the study of the symptoms produced by skatol, and for the determination of the lethal dose. The effect on the circulation and respiration was studied in cats and in one dog. Owing to the scarcity of skatol our studies were not extensive, but the results obtained were quite suggestive. The skatol used was of the highest purity, prepared by Kahl-

baum. Among the difficulties which presented themselves in making physiological studies with skatol was its insolubility in the ordinary solvents. After trying several we found that it was readily soluble in acetone. But this required careful controls, as we have shown elsewhere that acetone itself was toxic. In the experiments on frogs the skatol was dissolved in acetone, and by means of a hypodermic syringe was injected into the dorsal lymph sac. As shown in the table, 20 mgm. of skatol in

The toxicity of skatol and acetone. Experiments on frogs

FROG NUMBER	DATE	WEIGHT	ACETONE		SKATOL		REMARKS
			Total	Per 100 grams of frog	Total	Per 100 grams of frog	
		grams	*cc.*	*cc.*	*mgm.*	*mgm.*	
1	March 21–28	4)	0.50	1.02			In 30 minutes, equilibrium lost, next day recovered completely
2	March 21–22	65	0.35	0.48			Effect less marked than in "1", next day recovered completely
3	March 21–25	53	0.30	0.58	0.3	0.6	Prostration; equilibrium lost, frog recovered completely
4	March 21–27	54	0.40	0.75	0.4	0.8	Equilibrium lost in 3 minutes, recovery complete
5	March 25–27	46	0.20	0.44	20.0	44.0	Frog prostrated; breathing very slow; died four days after injection
6	March 23	42	0.30	0.70	30.0	70.0	Frog died in 30 minutes
7	March 28–29	42	0.40	0.95			Frog completely recovered next day
8	March 28	46	0.40	0.88	40.0	88.0	Frog died in 3 hours
1a	March 22–25	49	0.20	0.41	8.0	16.0	Reflexes exaggerated at first; breathing very slow; general prostration; on March 25 frog recovered
2a	March 22	65	0.40	0.62	16.0	25.0	In 30 minutes respiration stopped, opened body cavity—heart still beating (10 per minute)

0.2 cc. acteone given to a frog weighing 46 grams, or 43.4 mgm. skatol in 0.43 cc. acetone per 100 grams, caused symptoms of severe intoxication within a few minutes and the frog died four days later. A dose of 30 mgm. of skatol in 0.3 cc. acetone injected in a frog weighing 42 grams, or 70 mgm. skatol in 0.7 cc. acetone per 100 grams, was fatal within thirty minutes. A third frog weighing 46 grams which received 40 mgm. in 0.4 cc. acetone, or 88 mgm. skatol in 0.88 cc. acetone per 100 grams, died in three hours. The effects of acetone alone given to frogs used as controls showed that doses of 0.48 cc., 0.98 cc., and 1.02 cc. per 100 grams of frog were followed by recovery. Though symptoms were observed in the controls, they were less severe, and presented a striking contrast to those observed in frogs which received skatol with smaller amounts of acetone in proportion to body weight.

In experiments made to test the effect of skatol on the circulation and respiration the following results were obtained. Doses of 20 to 30 mgm. of skatol dissolved in 2 cc. of 50 per cent acetone were given rapidly by vein to two cats. The results on the cats were not very satisfactory as the solvent in the quantities given produced considerable depression of the circulation, but as shown in figure 1 this was greater when skatol was injected. When the skatol was given intravenously to a dog the results were more definite. Different amounts were injected, and were controlled by tests with acetone alone. In the first injection 60 mgm. of skatol in 1.2 cc. acetone were given very slowly at first, then more rapidly. Blood pressure fell from 150 to 100 mm. Hg, this low level persisting about 48 seconds. Respiration, on the contrary, was stimulated. The effect was not due to the acetone, since the injection of 1 cc. acetone produced only a slight transitory fall of blood pressure, although the injection was made twice as rapidly as in case of the skatol. Another injection (fig. 2) of 50 mgm. skatol in 1 cc. pure acetone made twenty minutes later, and injected in twenty-three seconds, produced a fall of blood pressure of about 50 per cent. Though complete recovery followed, it took nearly seven minutes before the blood pressure reached the same level as before the injection.
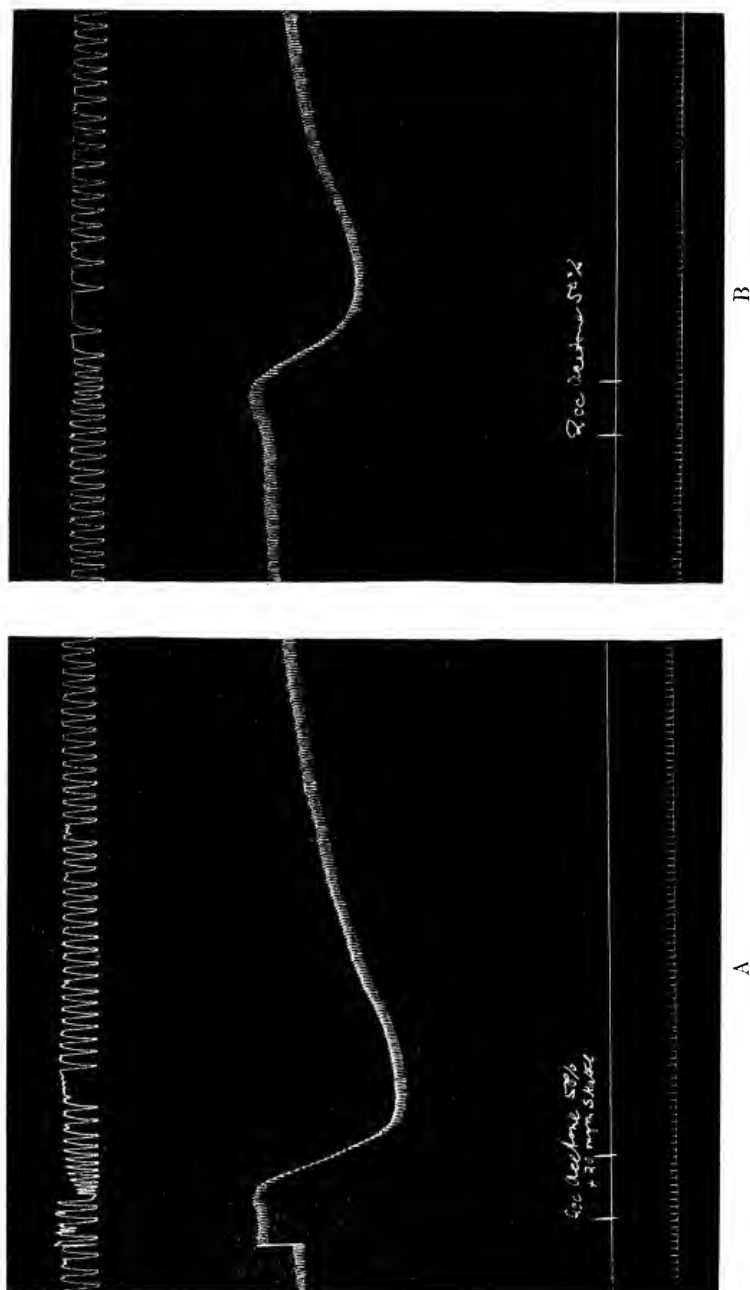
FIG. 1. CAT, WEIGHT 2.2 KILOS

Ether anesthesia. Fall of blood pressure in *A* after the injection of 20 mgm. skatol in 2 cc. 50 per cent acetone about 45 per cent. *B* after the same amount of acetone alone injected at the same speed fall of blood pressure about 30 per cent. Reduced one-half. Time in seconds.
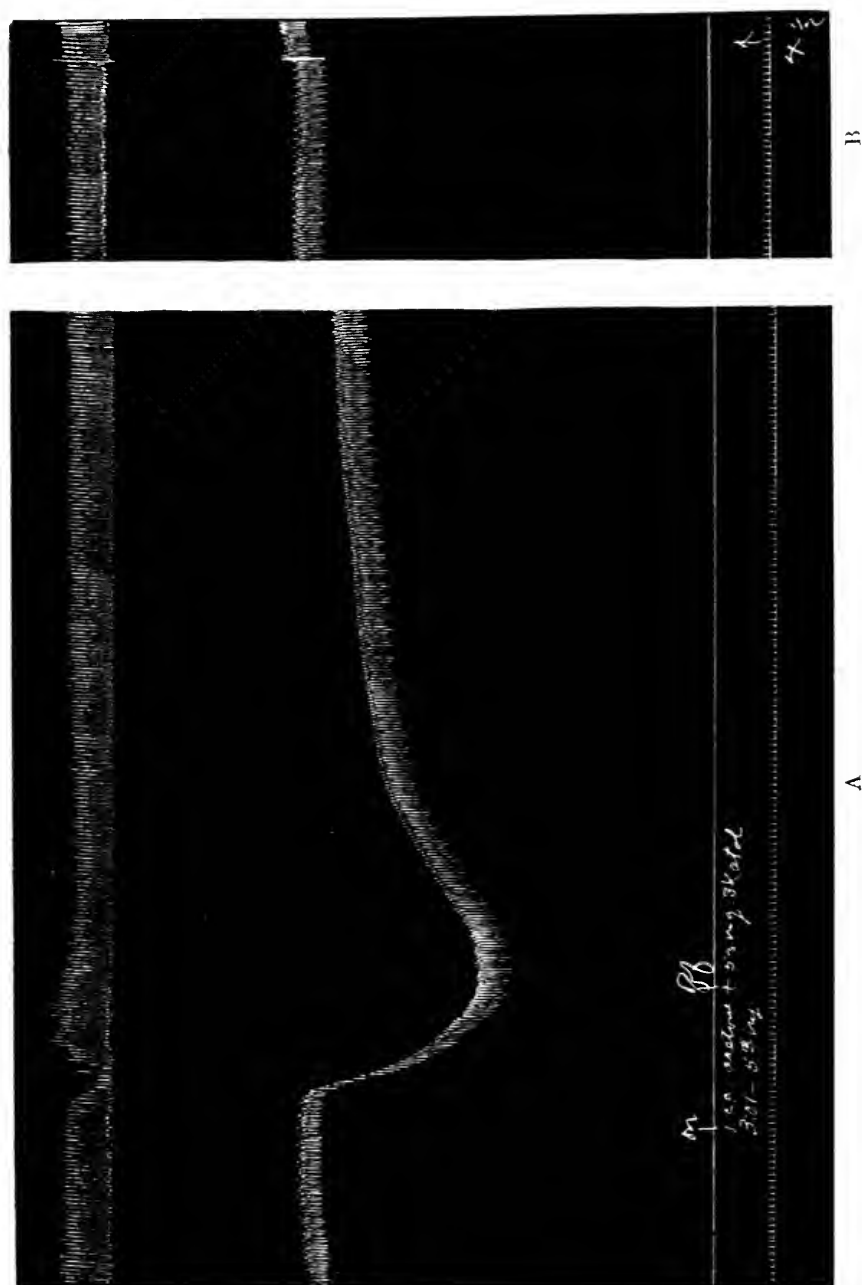
Fig. 2. Experiment 33. Injected 50 Mgm. Skatol in 1 cc. Acetone

Fall of blood pressure shown in A. Recovered in seven minutes, shown in B. Reduced two-thirds. Time in seconds

Respiration, was depressed for a brief period, and was followed by stimulation, but this was of short duration, as by the end of the injection respiration fully recovered.   The depression of the circulation produced by skatol was also shown when another injection was made of 80 mgm. skatol disolved in 0.8 cc. acetone. Blood pressure fell from 186 to 110 mm. Hg, or 76 mm., the speed of injection being much slower, almost half.
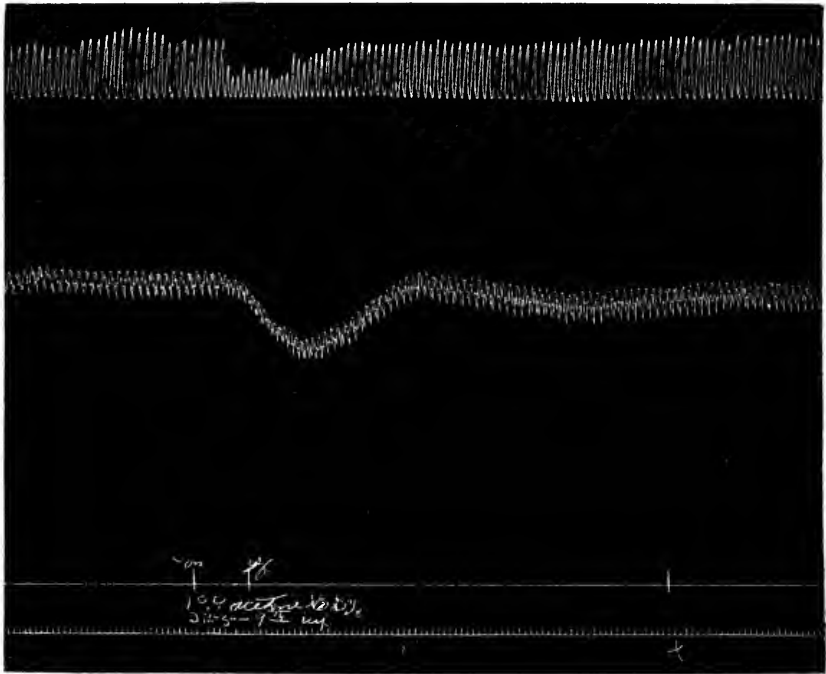
FIG. 3. EXPERIMENT 33.   MODERATE FALL OF BLOOD PRESSURE AFTER RAPID INJECTION OF 1 CC. ACETONE

Respiration depressed.   Reduced one-half.   Time in seconds

The slow recovery of the blood pressure which was observed in this case shows the depression produced by skatol is apt to persist.   Respiration, as in the previous injection, was temporarily stimulated, but was preceded by a brief period of apnea. When the effects of the injection of skatol are compared with those of 1 cc. of pure acetone, shown in figure 3, it will be noticed

that although the injection of 1 cc. of pure acetone was made in ten seconds, as compared with twenty-three and forty-eight seconds when skatol was injected, blood pressure fell only 34 mm. Hg (from 144 to 110 mm.), and recovered promptly. Another point of interest is that this control was made after acetone was given a number of times. As was stated by us (5) in a previous communication, acetone became more effective after repeated administration. Though this occurs more frequently in cats than in dogs, it also takes place in the latter when a considerable quantity of acetone has been introduced into the circulation. The observations recorded in the present report therefore justify the conclusion that skatol is a toxic substance causing depression of the circulation and of the central nervous system.

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SOME OBSERVATIONS ON THE ACTION OF MERCURY

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Examination of the literature on the action of mercury shows that a number of studies were made on the morphological changes which various compounds of the metal produced in the kidney and other organs. Reports were also published on the effect of some of its compounds on secretion and metabolism. Only one communication, however, could be found on its action on the heart, and another on its influence on the circulation and respiration. Dreser (1) investigated the action of mercury potassium thyocyanate on the isolated heart of the frog, and found that 3.8 mgm. mercury in 40 to 50 cc. of defibrinated blood of the ox produced cardiac paralysis, but the same or even larger amounts of mercury in the form of the hyposulphite had no effect. Experiments with mercury on intact animals were reported by Mueller, Schoelser and Schrauth (2). Various organic salts injected intravenously into cats caused, according to these observers, a fall in blood pressure and paralysis of respiration. The present report which is a continuation of studies on the action of heavy metals begun by one of us (3) several years ago, deals with observations on the influence of salts of mercury on the circulation and respiration in cats, dogs, and rabbits, and also includes experiments on the isolated heart of the turtle and of the frog.

METHODS

The experiments were carried out under complete anesthesia. Urethane or paraldehyde was used for cats, urethane for rabbits, and morphine-ether or paraldehyde for dogs. Mercury was given in the form of succinate, benzoate or acetate in a concen-

tration of 1:5000 in normal salt solution, and was injected into the femoral vein of cats and dogs, and into the jugular vein in experiments on rabbits. Blood pressure was recorded by means of a mercury manometer. Respiration was recorded by a Becker plethysmograph or by a method described by us (4) in a recent publication. The experiments on the isolated heart were made with different concentrations of the above salts, as well as of the bichloride, in Ringer's solution. The heart was perfused according to the method described by Salant and Livingston (5).

<center>EXPERIMENTS ON CATS</center>

Intravenous administration of the salts of mercury was usually followed by a moderate rise of blood pressure which occurred immediately after the injection and was probably due to the increased volume of fluid introduced into the circulation. Respiration became deeper, but there was no noticeable change in frequency. After an interval of a few minutes blood pressure fell rapidly at first, then more slowly, even when small doses of mercury were given. In some experiments, however, a sudden fall in blood pressure occurred which was associated with arrest of heart action lasting half to one minute, or even longer. As a rule, recovery took place, heart action being slow at first, but later it became more rapid, and the blood pressure was higher than before the depression set in. Very often the changes in the circulation were strikingly similar to those observed on stimulating the vagus. Respiration was depressed, but this occurred a few seconds after blood pressure fell. It may be observed in this connection that blood pressure sometimes recovered while the respiration was slow and superficial, or was altogether absent. The amount of mercury required to produce the changes in the circulation and respiration described varied from 2 to 4 mgm. per kilo. The effects were independent of the speed of injection, and they also occurred when the amounts were given in divided doses, thus indicating cumulation. The lethal dose, however, was much greater than that required to produce the disturbance of the circulation and respiration. A total of about 6 mgm. mercury per kilo may be regarded as the average dose necessary
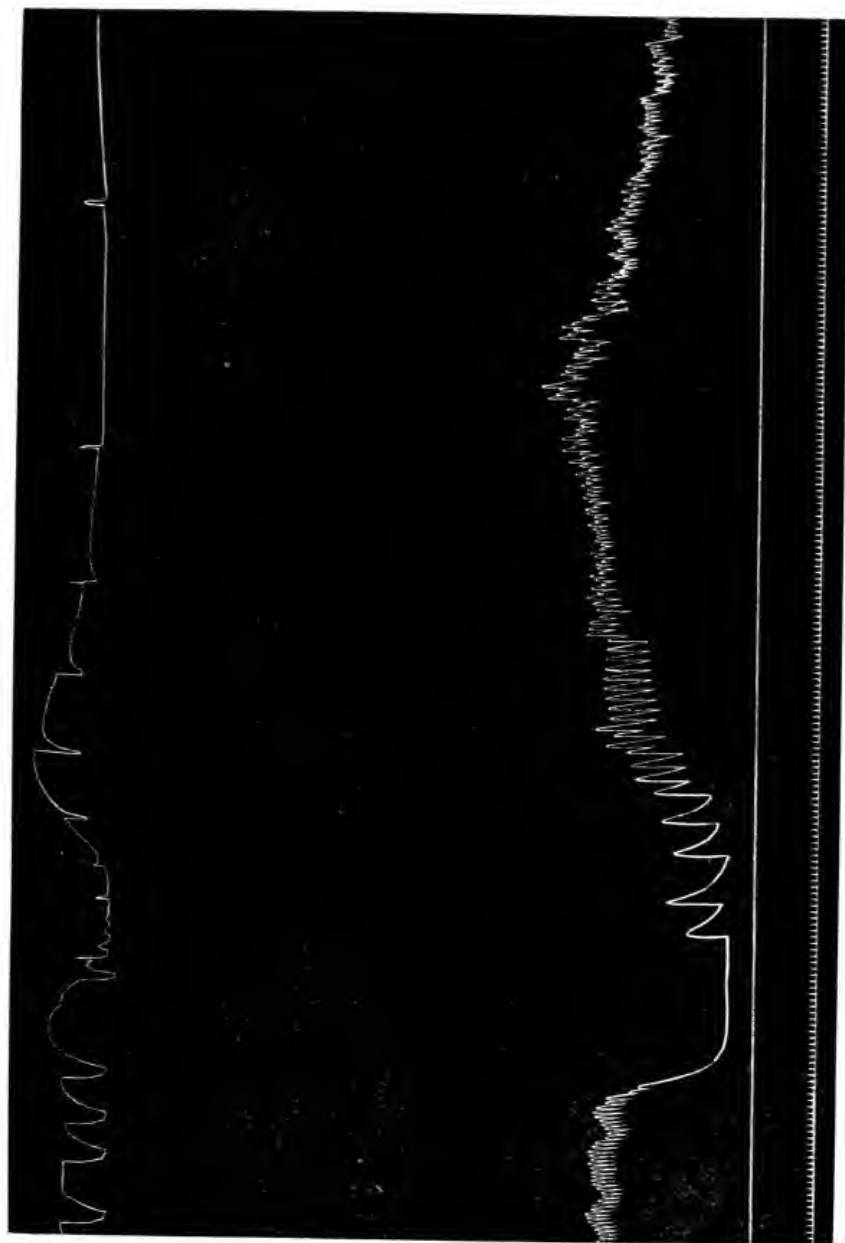
FIG. 1. EXPERIMENT 40.   CAT, WEIGHT 2.8 KILOS

Urethane anesthesia. Shows fall of blood pressure and cardiac inhibition produced by mercury benzoate after the injection of atropine sulphate. Reduced two-thirds. Time in seconds.

to cause paralysis of the circulation and respiration. But in some experiments the fatal doses were 8, 9, or even 10 mgm. mercury per kilo. In one case, on the contrary, a dose of 4 mgm. per kilo proved fatal. In every instance respiration was paralyzed before the heart which continued to beat for several minutes after respiration had stopped.

Illustrative experiments on the action of mercury in cats are presented.

Experiment 2. A cat weighing 2.5 kilos was given ether anesthesia. Forty cubic centimeters of mercury benzoate, or 8 mgm. mercury, were injected into the femoral vein in four minutes. No change was observed for some time. Four minutes after the injection blood pressure fell suddenly from 110 mm. to 50 mm. Hg, but recovered promptly. A second injection of the same amount of mercury, in the form of benzoate also, was made five minutes later. After a latent period of two minutes and fifty seconds blood pressure fell at first 50 mm. Hg, then descended until it reached the base line. Respiration was not affected after the first injection, but after the second injection paralysis occurred four minutes after the blood pressure began to fall.

Experiment 34. A cat weighing 3.3 kilos was given paraldehyde anesthesia. Three milligrams of mercury in the form of the acetate were injected into the femoral vein without producing any immediate effect. This amount was given, however, in two doses. The first injection of 1.5 mgm. mercury per kilo produced a slight rise of blood pressure, while the same dose repeated 6 minutes later caused, on the contrary, a fall of about 8 per cent. Respiration in both cases was somewhat increased in depth. A third injection was made six minutes after the second. When 2.4 mgm. mercury were now introduced, no change in respiration was observed, but blood pressure fell rapidly. The injection was nevertheless continued; 1.5 mgm. more were given, but before the injection was completed paralysis of the heart and respiration occurred, thus indicating cumulation.

Experiment 35. A cat weighing 3.2 kilos received ether-paraldehyde anesthesia. Both vagi were cut before the injections of mercury were begun. Twenty-five cubic centimeters of mercury succinate, or 5 mgm. mercury, were given at intervals of twelve to fifteen minutes, a total of 25 mgm. being injected. The first injection produced a rise of blood pressure which was preceded by a slight fall. Respiration was increased in depth, but the frequency was not noticeably changed.

In succeeding injections circulatory depression only was observed, and this increased with each dose. The maximum effect occurred after the fifth and final injection when the blood pressure fell from 110 to 50 mm. Hg, or about 60 per cent. Twenty minutes after this injection blood pressure showed a gain of only 10 mm. Hg, thus indicating that the depression produced tended to persist. The last injection only was followed by depression, while each preceding dose given caused an increase in depth of respiration without any considerable change in frequency. Cumulation, therefore, occurred likewise in this experiment.

Since the inhibitory effect on the circulation observed after the administration of the salts of mercury might be due to stimulation of the vagus mechanism, injections were made when both vagi were cut as well as into atropinized animals. It may be briefly stated that the changes produced in the circulation were similar to those observed when the salts of mercury were administered to cats in which the vagus mechanism was intact. We also made observations on the irritability of the vagus after injecting the various mercurials. The reaction to faradic stimulation was tested in a number of cats. Though the effect was doubtful in several cases, the response was sometimes distinctly less marked after the injection of the various compounds of mercury, thus indicating that the cardiac inhibition observed was not due to stimulation of the vagus mechanism.

EXPERIMENTS ON DOGS

Three dogs under morphine-ether anesthesia received benzoate of mercury intravenously. This was accompanied by a fall in blood pressure when a sufficient amount of the salt was introduced, and always took place several minutes after the completion of the injection. In one experiment respiration was considerably stimulated, while the blood pressure was lowered, but recovery occurred soon, the blood pressure being even higher than before. In the other two experiments blood pressure fell suddenly and respiration was paralyzed at the same time. This was observed in one experiment, when the total amount of mercury injected was 2.3 mgm. per kilo, in the other 5.6 mgm. per kilo, given in divided doses. The effects of mercury ben-

zoate when injected intravenously into dogs are illustrated in figure 2.   In this case the blood pressure fell about 33 per cent. It may be added that the depression of the circulation shown in this figure set in three minutes after the injection of 10 mgm. mer-
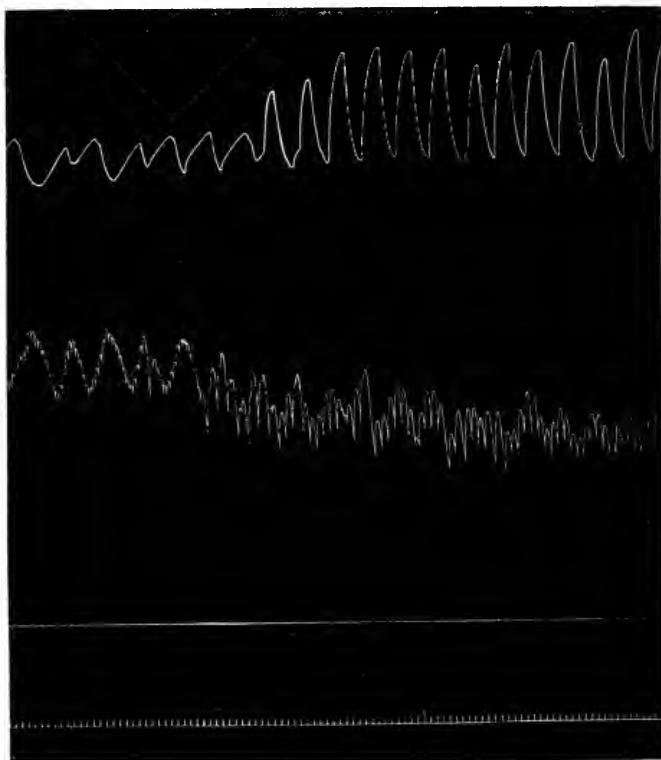
FIG. 2. EXPERIMENT 5.   DOG, WEIGHT 6 KILOS

Morphine ether anesthesia. Shows fall of blood pressure and increased depth of respiration which occurred three minutes after the intravenous injection of 50 cc. 1:5000 mercury benzoate (10 mgm. Hg).   Reduced one-half.   Time in seconds.

cury, the total amount previously injected being a little over 8 mgm. mercury per kilo.   Blood pressure continued to fall in this case until it reached a lower level than that shown in the figure, and continued without change for some time.   The next injection of 11 mgm. mercury in the form of benzoate made

several minutes later produced a similar effect. Respiration was not affected in this experiment. The stimulation shown in figure 2 was temporary and was soon followed by depression, frequency and depth being greatly decreased.

EXPERIMENTS ON RABBITS

The succinate of mercury was injected into the jugular vein of three full grown rabbits weighing about 2 kilos each. As in the experiments on dogs and cats, when a sufficient amount of an organic salt of mercury was administered, depression of the circulation and respiration occurred. In one experiment the first injection of 20 cc. of the succinate (4 mgm. mercury) produced a fall in blood pressure of about 15 per cent which persisted for some time after the injection, respiration being decreased in depth. A second injection of 14 cc., or 2.8 mgm. mercury, made five minutes later, caused a fall in blood pressure of 90 mm. Hg. In about four minutes the blood pressure recovered. The fall in blood pressure was in this case accompanied by struggling during which respiration became very violent for a brief period, and then subsided becoming very superficial. A third injection of 12.5 cc. of the succinate was made five minutes later, after the blood pressure had recovered and the respiration showed considerable improvement. This time blood pressure fell 55 mm. Hg. in about one minute, and after the injection was discontinued paralysis of the respiration occurred. It may be remarked that the formation of blood clots became so troublesome that the experiment had to be discontinued.

The action of mercury in the other rabbits indicated that 3 to 4 mgm. per kilo may be given intravenously without causing depression of the circulation or respiration, but the addition of about 1 mgm. per kilo may lower blood pressure and paralyze respiration. That cumulation also occurred in the rabbit was indicated by the fact that repeated injections of noneffective doses produced depression when the total amount introduced was sufficiently large.

THE INFLUENCE OF MERCURY ON THE ISOLATED HEART

Observations on the turtle and the frog heart were made with different concentrations of mercury in Ringer's solution. In most experiments the same heart was perfused several time for periods varying from one to thirty minutes, and in each case this was followed by Ringer's solution alone.

Experiments on the turtle heart. The highest concentration used, 1:10,000 mercury, has in all cases shown a very striking effect, even when the duration of the perfusion was short, a period of one minute being sufficient to cause arrest of the heart or to reduce its activity considerably. In some experiments, however, mercury, whether in the form of the chloride or the organic salts, caused moderate depression only. Heart action continued to be fairly strong during the perfusion, but was usually irregular and slower. There was little improvement, however, when the perfusion of the various preparations was discontinued. The injury produced by this concentration of mercury was permanent. In one experiment in which perfusion was continued for eighteen minutes, heart action steadily decreased in force until the contractions became too feeble to be recorded, and perfusion with Ringer's solution failed to restore the heart beat. In this experiment no irregularity of heart action was observed. When Ringer's solution containing mercury in a concentration of 1:100,000 was perfused, its immediate effect was seldom pronounced. But if the duration of the perfusion was sufficiently long, the injurious action of mercury could be observed. In three experiments in which the perfusion time was nine to sixteen minutes increased tonus was noticed in two, this being associated with a decreased amplitude as well as with a slight diminution in frequency which persisted in the after period when Ringer's solution alone was perfused. In the third experiment the contractions occurred in groups of 2 and 4, the amplitude being at the same time decreased. In five other experiments with the same concentration increased tonus was common in all as a result of the perfusion with any of the salts of mercury. This effect usually appeared in the form of well

FIG. 3. EXPERIMENT 44. TURTLE HEART

Perfused for one minute with mercuric chloride in Ringer's solution. Hg 1:10,000. *A*, shows feeble contractions after perfusion with mercuric chloride; *B*, shows condition of the heart fifteen minutes later when Ringer's solution alone was perfused. Reduced one-half. Time in five seconds.
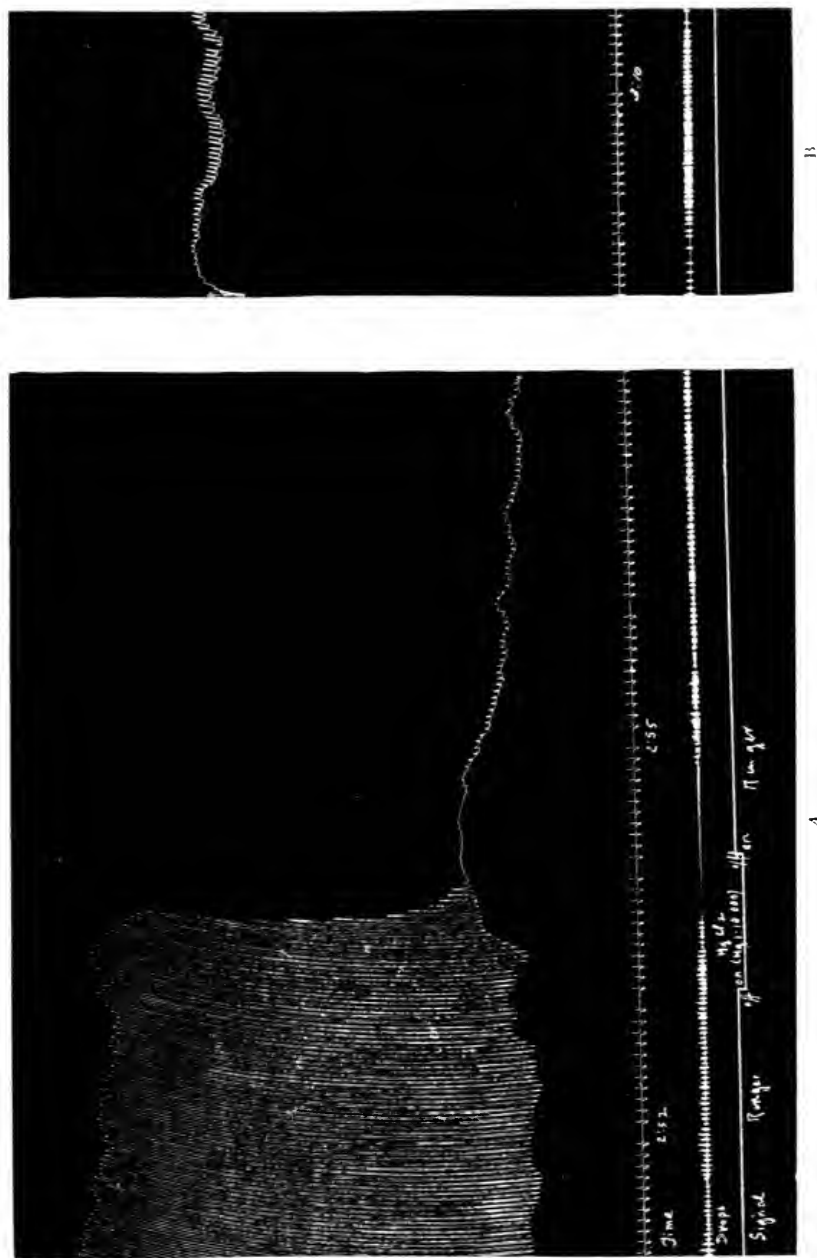
marked tonus waves four or five minutes after the heart has been exposed to the action of the salts. When the perfusion was continued further, other changes set in, such as irregular heart action (the contractions occurring in groups), diminished force and frequency, and partial heart block. Considerable improvement was sometimes observed, but complete recovery never occurred in any of our experiments when mercury was perfused through the turtle heart. It may be added that after repeated perfusions with the salts of mercury, in the same experiment, delirium cordis was a frequent occurrence. The effect of a still lower concentration was tested with the following results. When a concentration of mercury of 1:1,000,000 was perfused for five to eight minutes, delirium cordis was observed within five to nine minutes after perfusion was begun, and lasted several minutes to half an hour. In a series of experiments with mercury in a concentration of 1:10,000,000 similar effects were observed. Delirium cordis developed in one experiment ten minutes after the heart has been perfused with the salts. In another the contractions occurred in groups and were unequal in force. As in other experiments with mercury, the irregularity caused by it persisted for more than twenty minutes after perfusion was discontinued. Improvement, however, occurred later, and heart action became regular, though more frequent and the amplitude smaller. In a third experiment in which mercuric chloride was perfused for thirty minutes there was no delirium, but heart action became irregular, and the tonus waves which were observed before mercury was perfused became more pronounced. It is of interest to observe in this connection that in experiments with very dilute solutions of mercury the abnormal changes often appeared after a comparatively short latent period.

Experiments on the frog heart. The same compounds were employed as those used for testing the action of mercury on the turtle heart. The response of the frog heart to mercury was found to be different in several respects from that observed in the turtle hearts. It will be recalled that in the latter very dilute solutions—1:1,000,000 or even 1:10,000,000—mercury were still effective. This was not the case in the frog heart,
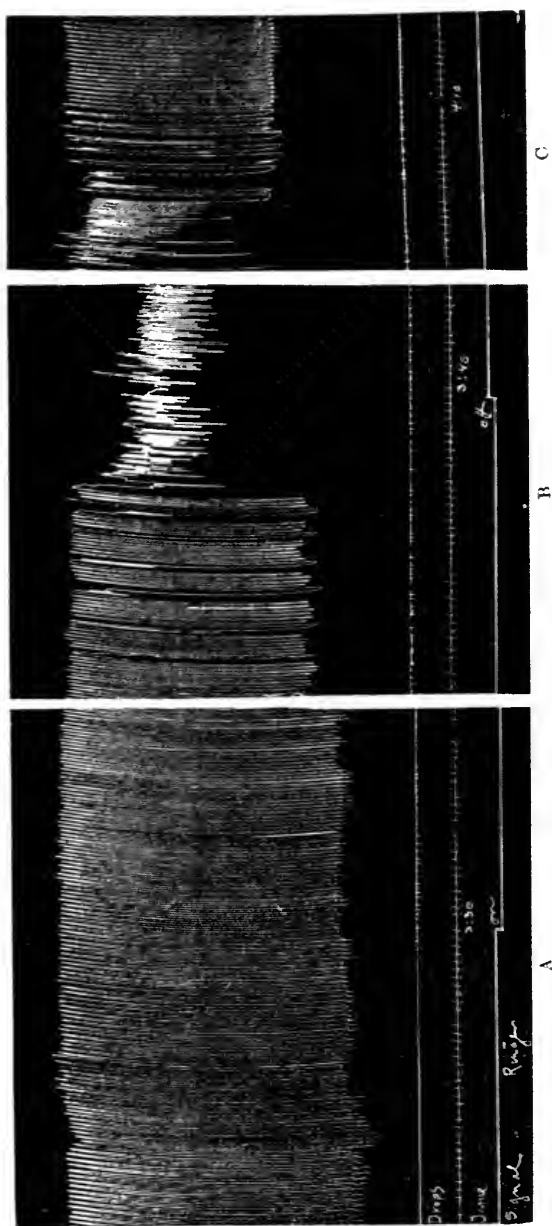
FIG. 4. EXPERIMENT 55. TURTLE HEART PERFUSED WITH MERCURIC CHLORIDE (Hg 1 : 1,000,000), FOR TEN MINUTES

Heart action became irregular when perfused with mercuric chloride for ten minutes. Two minutes later as shown in B, delirium cordis developed which lasted about one-half hour; C shows recovery. Reduced one-half. Time in five seconds.

as a concentration of mercury of 1:1,000,000 failed to produce any visible changes in heart action. Rarely was any effect obtained even when the concentration of mercury of 1:100,000 was perfused through the heart. Evidence of marked injury was first observed when mercury in a concentration of 1:50,000 was used. Moderate depression occurred when the period of perfusion lasted five minutes, and when this was repeated five
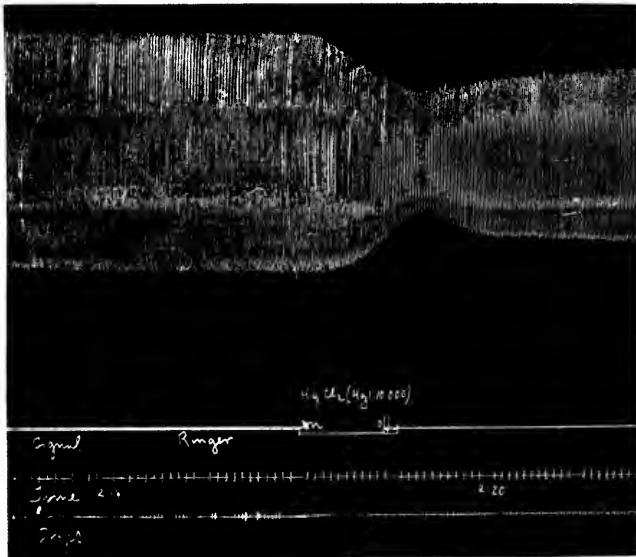
Fig. 5. Experiment 79. Frog Heart Perfused for One Minute with Mercuric Chloride in Ringer's Solution (Hg 1:10,000) Shows Depression

Improvement of heart action occurred when perfused with Ringer's alone, but complete recovery never occurred. Reduced one-half. Time in five seconds.

minutes later, arrest of heart action took place which lasted for four minutes. In other experiments the force of the heart was decreased, being reduced sometimes to a small fraction of its original magnitude. It may be added that though some improvement occurred on returning to Ringer's solution, heart action was not completely restored. With still greater concentrations of mercury the changes were more pronounced. Particularly striking were the results obtained when the frog heart was perfused with
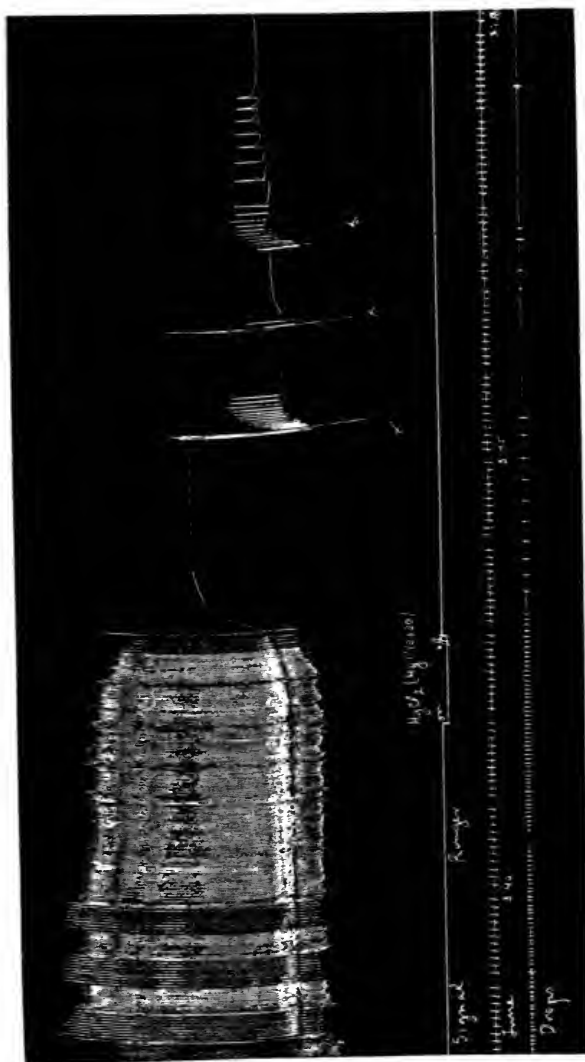
FIG. 6. EXPERIMENT 80. FROG HEART PERFUSED FOR ONE MINUTE WITH MERCURIC CHLORIDE
IN RINGER'S SOLUTION (Hg 1:10,000)

Heart stopped, ventricles first, then auricles. Heart distended with fluid was gently squeezed
as indicated in figure by mark x, but no recovery occurred. Heart previously perfused with
atropine sulphate in Ringer's solution. Reduced one-half. Time in five seconds.

the salts of mercury in a concentration of 1:10,000. The action observed was of two types. In some experiments perfusion with the salts of mercury produced a steady decrease in the force of the heart beat, frequency being slightly affected or not at all. This type of change was noticed in very few experiments, but in a large number of cases perfusion with this concentration (and also with more dilute solutions) for a period of one minute or longer caused sudden arrest of heart action, the ventricles stopping first, then the auricles. Very often the auricles would continue to beat several minutes after the ventricles stopped. In a number of experiments perfusion of the frog heart with a solution of a mercuric compound caused disturbance of rhythm, the auricles beating more frequently than the ventricles. Little or no improvement occurred when perfusion with mercury was stopped and Ringer's solution alone was perfused instead. Finally, it may be added that delirium cordis so frequently observed in the turtle heart never occurred in the frog heart on perfusion with mercury salts.

DISCUSSION

Though the action of mercury varied considerably in different animals, these experiments nevertheless demonstrated that it exerted a powerful effect on the circulation as well as on the respiration. Small quantities of mercury, it will be recalled, produced a very considerable, and sometimes persistent fall in blood pressure in cats, dogs, and rabbits. And even when a small dose was given repeatedly cardiac paralysis occurred. The reaction to mercury in cats was in many cases quite different from that observed in other animals. Sudden inhibition of the heart lasting in some instances a minute or longer, and followed by a prompt recovery, was observed in cats alone. These changes were not due to stimulation of the vagus mechanism, for they occurred after section of both vagi and even after the injection of atropine. It may be observed that the reaction of the vagus to faradic stimulation indicated that its irritability was, on the contrary, decreased when the salts of mercury were injected, and it may be added that the sudden arrest of heart action like-

wise occurred in the isolated heart of the frog when perfused with mercury, and that this effect was also observed in atropinized specimens.   It is evident, therefore, that the vagus mechanism was not concerned in the cardiac inhibition produced by mercury.

The reaction of the isolated heart to mercury, as observed in our experiments, indicated that the response was, to a certain extent similar in the frog heart and in the turtle heart.   Both were depressed when perfused with concentrations of mercury of 1:10,000 and 1:50,000 in Ringer's solution, but the turtle heart reacted also to Ringer's solutions containing mercury in a dilution of 1:1,000,000 and even 1:10,000,000, whereas a response was seldom obtained in the frog heart to concentrations of 1:100,000, and as noted above, delirium cordis was observed in the turtle heart only.

Some differences were also observed in the potency of the organic salts and the bichloride.   Attention may be called in this connection to the comparative action of mercury and other heavy metals.   In experiments on the isolated frog heart by Salant and Connet (6) it was found that the salts of zinc and cadmium were the most toxic of all the heavy metals reported. As judged by the effect on the frog heart the action of mercury is about the same as that of zinc.

Finally it may be pointed out that the results we obtained with the compounds we employed differed from those reported by previous investigators.   Dreser's observations (1) on the isolated heart, though not strictly comparable with our experiments, indicate that the toxicity of mercury is less than that found in our tests with the different salts, especially with the bichloride.

According to Mueller, Schoeler and Schrauth (2) a pronounced fall in blood pressure and paralysis of respiration was produced in cats after the intravenous injection of 37.5 mgm. of sodium oxymercury propionate per kilo which is much greater than effective doses that produced similar results in our experiments. But the more recent observations of Kolmer, Schamberg and Raiziss (7) on the toxicity of various organic salts of mercury showed that the minimum lethal dose given intravenously into

rabbits varied between 3.5 and 6.7 mgm. mercury per kilo.   Their findings agree very closely with the results we obtained in experiments on rabbits.

SUMMARY

1. The intravenous injection of the acetate, succinate and benzoate of mercury into cats, dogs and rabbits produced a sudden fall in blood pressure which was very marked and persistent. Depression and later paralysis of respiration also occurred.

2. Cardiac inhibition was produced by the intravenous injection of the salts of mercury in cats, but not in dogs or in rabbits.

3. Decreased irritability of the vagus was observed in cats after the intravenous injection of the organic salts of mercury.

4. That the fall in blood pressure after mercury was of cardiac origin was shown by observations on changes in the volume of the kidney.

5. Perfusion of the turtle heart with the different salts of mercury produced cardiac depression, irregularity and delirium cordis. Concentrations of one part of mercury to one million parts of Ringer's solution, and even one to ten million, were effective.

6. The frog heart was more resistant to mercury than the turtle heart. No delirium cordis was observed.

7. The action of mercury is cumulative.

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CHANGES WITH ADVANCING AGE IN THE RESISTANCE OF THE ALBINO RAT TO ARSENIC

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In the preceding paper (1) the toxic dose of arsenic trioxide when administered subcutaneously to the normal, over 200-day old, mature, non-pregnant, female albino rat was found to be 8 mgm. per kilo of body weight. In repeating the experiment upon younger rats it was found that the average fatal dose did not conform with this finding. We therefore decided to repeat the experiment as far as arsenic trioxide was concerned upon a series of rats of different ages in order to determine what effect, if any, the age of the rat had in determining its resistance to poisoning by arsenic.

The arsenic trioxide solution used was prepared as described in the first report, e.g., a 0.2 per cent solution was made and sterilized before administration. The animals used in these studies were normal, non-pregnant, female albino rats. There were three groups, each of different ages. The first group consisted of young rats 60 to 90 days of age; the second consisted of rats 120 to 150 days old, and the third group was made up of animals 210 to 240 days old.

The diet was uniform for all series in order that this possible source of variation in resistance to poisoning should be eliminated (2). The procedure for the administration of the arsenic solution was identical with that previously described.

The results of the tests are given in the accompanying table. It is evident that with advancing age there is a progressive diminution in the resistance of the albino rat to fatal poisoning by arsenic.

331

The influence of age on the resistance of female albino rats to death from arsenic poisoning

As₂O₃ injected per kilo	GROUP 1, 60–90 DAYS OLD		GROUP 2, 120–150 DAYS OLD		GROUP 3, 210–240 DAYS OLD	
	Body weight	Remarks	Body weight	Remarks	Body weight	Remarks
mgm.	*grams*		*grams*		*grams*	
4					161	Lived
						Lived
5			145	Lived	192	Lived
			124	Lived		
6	119	Lived	126	Lived	162	Lived
			127	Lived	210	Lived
					181	Lived
7	119	Lived	133	Lived	188	Lived
	115	Lived	135	Lived	176	Lived
					223	Died over night
8	120	Lived	142	Lived	197	Lived
	106	Lived	149	Lived	199	Lived
	122	Lived	130	Lived	184	Lived
	107	Lived	153	Lived	217	Died over night
	127	Lived	153	Died over night	180	Died over night
					197	Died over night
					189	Died over night
9	105	Lived	141	Lived	186	Died over night
	117	Lived	168	Lived	186	Died over night
	100	Lived	130	Lived	212	Died over night
	109	Lived	117	Died over night	211	Died over night
			126	Died over night	184	Died over night
			171	Died over night	217	Died over night
					204	Died over night
10	123	Lived	132	Died over night	200	Died over night
	123	Lived	127	Died over night	175	Died over night
	137	Lived	142	Died over night	170	Died over night
	106	Lived	156	Died over night	197	Died over night
	115	Lived	153	Died over night	202	Died over night
	101	Lived			196	Died over night
	101	Lived				
	111	Died over night				

As$_2$O$_3$ IN-JECTED PER KILO	GROUP 1, 60–90 DAYS OLD		GROUP 2, 120–150 DAYS OLD		GROUP 3, 210–240 DAYS OLD	
	Body weight	Remarks	Body weight	Remarks	Body weight	Remarks
mgm.	grams		grams		grams	
11	103	Died over night				
	123	Died over night				
	100	Died over night				
	111	Died over night				
12	110	Died over night				
	108	Died over night			176	Died over night
	100	Died over night				
13	120	Died over night				
	108	Died over night				

In the young animals of 60 to 90 days of age the fatal dose is seen to be 11 mgm. per kilo of body weight; but one of this series succumbing to the lesser amount of 10 mgm. per kilo.

In the group of rats ranging from 120 to 150 days in age the fatal dose for the average animal is 9 milligrams or over; but one of this series dying from the smaller amount of 8 mgm. per kilo.

In the group of old rats of ages from 210 to 240 days it is seen that 8 mgm. can be safely considered as the dose which is fatal to the average rat in this series; but one dying from the lesser dose of 7 mgm. per kilo. This confirms the findings of the preceding paper for animals of this age.

Stating the results in another way: None of the youngest rats survived a dose of 11 mgm. or over of arsenic trioxide per kilo of body weight: none of the middle aged rats survived the dose of 10 mgm. or over and 50 per cent of those receiving 9 mgm. per kilo died: and none of the old rats survived 9 mgm. per kilo and 60 per cent of them died from the effects of 8 mgm. per kilo.

All of the deaths occurred within twenty-four hours of the time of administration of the poison.

In view of these results on 80 rats we feel justified in concluding that changes due to age are potent factors in the resistance of the albino rat to arsenic poisoning.

Comparing these findings with observations reported for man there is at once noticed a lack of recognition of the early statement of Fowler (3) in his original monograph on the use of arsenic, that "I observed that children were less apt to suffer from the medicine than adults," referring of course to the effect following the use of the solution bearing his name. From the point of view of general biology these differences in susceptibility to poisons dependent on age have been all too little stressed, MacNider (4) standing almost alone in pointing this out as a subject worthy of investigation. His observations on the influence of age on the resistance of animals to poisoning by uranium nitrate are supported in principle by the findings reported in this paper.

Just as the difference in resistance of man and rat to arsenic can be attributed in large part to a difference in the respective rates of protein metabolism, so is it probable that the same factor comes into play in bringing about the differences in susceptibility observed in rats of different ages.

Although we have not data concerning the relative metabolic rates of young and older rats, from the fact that the protein metabolism of the rat runs closely parallel with that of man in its essentials as reported by Folin and Morris (5), and because the processes of growth for living organisms have been found by Loeb (6), Robertson (7), Ostwald (8), Brody (9) and others to follow the same general fundamental law, it is not improbable that the metabolic rate of young animals is greater than that of old as has been indicated by Benedict (10) in his studies of the energy requirements of children. Moreover as Benedict and Talbot (11) have pointed out, there is strong evidence that the active mass of the protoplasmic tissue determines the fundamental metabolism. With advancing age the mass of active protoplasmic tissue relative to the organism as a whole decreases. In this connection Doctor Donaldson has kindly pointed out to us that the viscera, as representative of a particularly active mass of protoplasmic tissue, decrease in their percentage relation to the body weight with advancing age, as can be seen from tables 73 and 74 in his book "The Rat" (12). These results

and our data are in agreement with Jackson's observations for the percentage weight of the viscera at similar ages (13). Moreover the blood volume decreases with advancing age. All these structural changes signify a decreased rate of metabolism and a decreased facility for getting rid of noxious agents and the damage caused by them. This is a factor of no mean importance in determining the resistance capacity of the organism.

SUMMARY AND CONCLUSION

It is evident from this study that with advancing age there is a progressive diminution in the resistance of the albino rat to death from arsenic poisoning. A dose of 11 mgm. or over of arsenic trioxide is fatal to young rats of 60 to 90 days of age. By the time the animals have reached the age of 120 to 150 days a dose of 9 mgm. is fatal. When they are 210 to 240 days old, 8 mgm. is the amount which produces death within 24 hours in the average animal.

These differences in susceptibility with age are attributable in large part to differences in metabolic rates.

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THE RELATIVE TOXICITY OF GERMANIUM AND ARSENIC FOR THE ALBINO RAT

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The element germanium has a certain historical interest in the development of the science of chemistry in that it is one of the three substances the properties of which were foretold by Mendelejeff (1) by the aid of the periodic law. Its name is derived from the fact that it was discovered by Winkler (2) in Germany in the silver ore at Freiberg. The chemical reactions and compounds of germanium somewhat resemble tin and the other members of the tin group, arsenic and antimony On account of this similarity and because germanium lies next to arsenic in the periodic system it seemed worth while to determine whether the element exhibits a similar toxicity towards the living organism as does arsenic, or whether it is relatively non-toxic as is tin.

In the experiments which are here reported there was used a 0.4 per cent solution of germanium dioxide in Ringer's solution. The oxide was a sample of the chemically pure compound prepared and used by Muller (3) in his determination of the atomic weight of the element. For the testing of the toxicity of the arsenic there was prepared a 0.2 per cent solution of arsenic trioxide in Ringer's solution. Both of these stock solutions were sterilized before administration.

The animals used in the study were mature, over 200 days old, non-pregnant female albino rats. As Hunt (4) has reported that the resistance of the animal organism to poison varies with the diet, it should be noted that the diet for the animals used here was uniform throughout.

337

An attempt was made to administer the oxides by mixing them with the food; but it was soon found that quantitative dosage by this method was not attainable. Consequently the compounds were given by subcutaneous injections. Each animal was weighed immediately before the injection of the solution of the oxide, which was accomplished while the' rat was under light ether anesthesia. The dosage in each case was graduated according to the weight of the animal and the amount that it was desired to administer per kilo of body weight.

Three series of animals were used for the determination of the toxicity of arsenic trioxide. The first, a preliminary series, covered a wide range of dosage in order to determine approximately the lower limits for the fatal dose. The second and third series were within narrower limits. In the study of the effect of germanium but two series of rats were used because it was found that the germanium oxide is quite non-toxic even in relatively enormous doses. The results are given in the accompanying tables.

It is seen that germanium differs markedly from arsenic with respect to its toxicity for the albino rat in that amounts of the former up to 180 mgm. per kilo of body weight produce neither death nor apparent evidences of harmful effect. Hence in so far as toxicity for the organism is concerned germanium resembles tin more than it does arsenic. This is physiological evidence that germanium is more closely allied to the tin group than to the arsenic group with regard to its position in the periodic system.

The results of the injections of arsenic trioxide show (table 1) that by and large 8 mgm. per kilo of body weight can be considered as the lethal dose for the average adult female albino rat.

All of the rats which were injected with arsenic and which did not die, later developed areas of necrosis at the site of the injection. No necrosis followed the injection of the germanium dioxide.

Comparing these results with arsenic with the reports of other investigators on studies of arsenic toxicity on lower animals we have been unable to find any references to work done on

TABLE 1

The results of the subcutaneous injections of arsenic trioxide solution

RAT—♂ NUMBER	BODY WEIGHT	As₂O₃ INJECTED	As₂O₃ PER KILO BODY WEIGHT	REMARKS
Preliminary series				
	gm.	mg.	mg.	
1	170	0.23	1.3	Lived
2	159	0.41	2.6	Lived
3	161	0.65	4.0	Lived
4	162	0.99	6.0	Lived
5	223	1.56	7.0	Died over night
6	149	1.19	8.0	Lived
7	212	1.91	9.0	Died over night
8	200	2.00	10.0	Died over night
9	176	2.11	12.0	Died over night
10	225	3.60	16.0	Died over night
11	173	5.64	32.0	Died over night
Second series				
1	181	1.09	6.0	Lived
2	209	1.26	6.0	Lived
3	175	1.23	7.0	Lived
4	188	1.32	7.0	Lived
5	197	1.58	8.0	Lived
6	199	1.60	8.0	Lived
7	217	1.74	8.0	Died over night
8	186	1.67	9.0	Died over night
9	186	1.67	9.0	Died over night
10	175	1.75	10.0	Died over night
11	170	1.70	10.0	Died over night
Third series				
1	184	1.48	8.0	Lived
2	180	1.44	8.0	Died over night
3	191	1.58	8.0	Died over night
4	189	1.51	8.0	Died over night
5	211	1.90	9.0	Died over night
6	184	1.66	9.0	Died over night
7	217	1.95	9.0	Died over night
8	204	1.84	9.0	Died over night
9	197	1.97	10.0	Died over night
10	202	2.02	10.0	Died over night
11	196	1.96	10.0	Died over night

the albino rat.   For the rabbit, guinea-pig, dog, cat and mouse, Willberg (5) reports the maximum tolerated dose of potassium arsenite to be 8 to 10 mgm., 9 mgm., 7 mgm., 5 to 6 mgm., and 15.6 to 17.6 mgm. respectively per kilo of body weight.   Brouar-

TABLE 2

The results of the subcutaneous injections of germanium dioxide solutions

RAT—♀ NUMBER	BODY WEIGHT	GeO₂ INJECTED	GeO₂ PER KILO BODY WEIGHT	REMARKS
First series				
	gm.	*mg.*	*mg.*	
1	177	0.17	1	Lived
2	164	0.32	2	Lived
3	162	0.81	5	Lived
4	167	1.67	10	Lived
5	156	3.12	20	Lived
6	134	5.86	40	Lived
7	136	10.88	80	Lived
8	190	34.30	180	Lived
Second series				
1	131	1.31	10	Lived
2	117	1.17	10	Lived
3	100	1.00	10	Lived
4	117	1.17	10	Lived
5	105	2.10	20	Lived
6	107	2.14	20	Lived
7	95	1.90	20	Lived
8	109	2.19	20	Lived
9	98	3.94	40	Lived
10	117	4.68	40	Lived
11	95	3.82	40	Lived
12	123	4.92	40	Lived
13	134	10.72	80	Lived
14	150	12.00	80	Lived
15	132	10.56	80	Lived
16	120	9.60	80	Lived

del (6) observed that a dose of 10 mgm. per kilo of arsenious acid is fatal for the rabbit, while a dose of 13 mgm. per kilo when injected subcutaneously is fatal to the guinea-pig.   Towles (7) working with mice reports the minimal lethal dose of potas-

sium arsenite as from 13 to 15 mgm., per kilo body weight when injected subcutaneously.

When we come to compare our results with those scattered throughout the literature with respect to the fatal dose for man we are confronted with the lack of experimental data. Nevertheless Joachimoglu (8) has given the fatal dose for man as lying between 0.1 and 0.2 grams. This is equivalent to 1.6 to 3.2 mgm. per kilo body weight of a 60-kilo man. In the basis of these values the rat is from two to four times as resistant to arsenic as is man.

This difference in resistance to fatal poisoning by arsenic of the two species is probably due to differences in the rates of the processes concerned in protein metabolism. For calculations made from the values published by Folin and Morris (9) for the protein metabolism of the albino rat as expressed by the urinary nitrogen excretion show that the nitrogenous excretion of the normal rat is from two to four times that of the average man, using the values published by Folin (10) in 1913 as the basis of the nitrogenous metabolism of the latter: This is further supported by the observations of Ringer and Murrell (11) which demonstrate that arsenic is a protoplasmic poison and destructive of nitrogenous tissue, and the fact that the nitrogenous metabolism of the rat is in many essentials entirely comparable with that of man with respect to the partition of the urinary nitrogen (9).

SUMMARY AND CONCLUSIONS

A comparison of the relative toxicity of germanium dioxide and arsenic trioxide for the albino rat shows that the former can be administered subcutaneously in doses up to 180 mgm. per kilo of body weight of the experimental animal with no apparent harmful effects. The latter usually produces a fatal result when similarly given to mature, non-pregnant female animals in the ratio of 8 mgm. per kilo of body weight.

Moreover the injection of arsenic trioxide solutions is followed by marked necrosis and sloughing at the point of injection, which phenomena are not sequelae of the injection of germanium dioxide solutions.

It is therefore evident that germanium does not possess the toxicity for the living organism such as is exhibited by arsenic.

It would appear from these results that the albino rat is more resistant to poisoning by arsenic than is man. Correlated data indicate that this difference is due to the difference in the degree of the protein metabolism of the two species.

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THE HEMOLYTIC PROPERTIES OF ARSPHENAMINE AND FIFTEEN ALLIED COMPOUNDS[1]

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The arsines are the only compounds of arsenic that had been shown to possess hemolytic properties until Schamberg, Kolmer, Raiziss and Weiss (1), and Kolmer and Yagle (2) called attention to the fact that arsphenamine is hemolytic; but insignificantly so, if care is taken to dissolve it in salt solution of such a strength that the resulting solution will be isotonic when the arsphenamine hydrochloride is changed to the disodium salt by the addition of sodium hydroxide.

The present work was undertaken in the hope of finding a more convenient test object than the rat for the determination of the toxicity of various preparations of arsphenamine.[2] The

[1] This is the fifth of a series of studies on the properties contributing to the toxicity of arsphenamine being made under a grant from the United States Interdepartmental Social Hygiene Board to the Harvard Medical School, the work being under the general direction of Doctor Reid Hunt.

[2] It may be of service to mention that the effect of samples of the drug of varying toxicity were tried on the following objects with uniformly negative results as far as the relation between toxicity and action in a quantitative manner is concerned.

1. The effect of solutions of arsphenamine on the catalase of frog's muscle according to the method of Santesson (Skand. Arch. f. Physiol., 1914–15, xxxii, 405).

2. The effect on the fermentation of sugar by yeast.

3. Experiments with B. subtilis, B. typhosus, and Staph. aureus, using a method essentially like that of determining the phenol coefficient.

4. Similar experiments with Infusoria, especially Paramoecium and Colpidia.

5. Injections into the anterior lymph sac of frogs.

6. Adding known concentrations to water in which Fundulus was swimming.

7. Amoeba in pure culture.

8. Frog spermatozoa, in the attempt to show the presence of phenyl hydroxylamine.

9. H-ion concentration of both alkaline and acid solutions. These were found to vary only within extremely narrow limits, the former between pH 9.6 and 10.0, averaging 9.9, the latter between 4.4 and 4.8, averaging 4.6.

343

hemolytic power of various samples of arsphenamine (as disodium salt) ran roughly parallel to the toxicity as determined by intravenous injections in rats.

The test-object was a "five per cent suspension" of washed sheep's corpuscles in 0.85 per cent salt solution.[3] The arsphenamine solutions were made by adding the calculated amount of N/1 sodium hydroxide to a freshly prepared solution of the hydrochloride. This was diluted to exactly two per cent in glass distilled water. To the solution of the disodium salt, so prepared, an equal quantity of 1.36 per cent salt solution was added, making a one percent solution in 0.68 per cent salt solution. Such a solution is said by Kolmer and Yagle to be exactly isotonic. To a series of five tubes was added 0.3, 0.4, cc., etc., of this solution and enough physiological salt solution to make the total volume in each tube 1 cc. Then 1 cc. of a 5 per cent suspension of red cells was added to each tube and the tube immediately inverted to mix the contents as rapidly as possible. As soon as the dilutions were completed, the tubes were placed, unstoppered, in the incubator at 37°C. for an hour. They were then put in the ice-box at 4°C. over night and the results read the following morning. We found that comparable results could be obtained if the tubes were left in the ice-box from twelve to twenty-four hours. We have indicated the degree of hemolysis by making the readings from single plus to four plus, showing respectively, slight, definite, marked, and complete hemolysis. Great care was taken to prevent oxidation in the preparation of the arsphenamine solutions and in their subsequent handling, both by reducing agitation to a minimum and by using only freshly prepared solutions.

The first experiments consisted in a comparison of the hemolytic activity of samples prepared by different methods and of varying toxicity.[4] The toxicity was, in this group of experi-

[3] These corpuscles were obtained from the State Wassermann Laboratory through the kindness of Dr. W. A. Hinton.

[4] Nearly all of the preparations used in these experiments were made by Mr. W. G. Christiansen in this laboratory. The toxicity determinations on rats were made by Dr. Reid Hunt.

ments, nearly always of the second type. described by Hunt (3); namely, the toxicity variations produced by changes in the conditions under which the nitro group is reduced in the preparation of the sample (4). The hemolytic acitivity of these samples varied roughly as did their toxicity (cf. table 1) but this was not constant nor delicate enough to be used as a compara-

TABLE 1

DATE	SPECIMEN* NUMBER ARSPHENAMINE	TOXICITY †	HEMOLYSIS REACTION					PREPARATION OF SAMPLE
			1 per cent disodium arsphenamine solution					
			0.3 cc.	0.4 cc.	0.5 cc.	0.6 cc.	0.7 cc.	
4/12/21	42	140	0	0	0	+	+	By reducing amino group with hypophosphorous acid‡
4/13/21	42	140	0	0	0	0	0	
4/14/21	42	.140	0	0	0	+	++	
4/12/21	173	120–130	0	0	0	++	++	From amino acid
4/13/21	173	120–130	0	0	0	0	0	
4/14/21	173	120–130	0	0	0	0	++	
4/12/21	123	100	0	0	+	++	+++	By standard method
4/13/21	123	100	0	0	0	0	0	
4/14/21	123	100	0	0	0	0	0	
4/12/21	179	70	++	+++	++++	++++	++++	By standard method with slow reduction of the nitro group
4/13/21	179	70	+	+	+++	++++	++++	
4/14/21	179	70	0	0	++	+++	++++	

* When not in use solutions (alkaline 1 per cent in 0.62 per cent salt solution) were kept in the ice-box at 4°C.

† By "toxicity" in this and subsequent tables is meant the highest dose in milligrams per kilo tolerated by rats on intravenous injection.

‡ Christiansen, Jour. Amer. Chem. Soc., 1920, xlii, 2402.

tive test in the same manner as the rat test. Table 1 also shows the effect of standing in the ice-box on the hemolytic power of solutions of the disodium salt; such a decrease in hemolytic power is always seen in old solutions, and the same result can be obtained by agitating the solution. In this connection, it should be noted that the "arsenoxide" (m-amino-p-hydroxy-

phenylarsenious oxide) separately prepared, is found to be non-hemolytic in the dilutions used.

It was then found that warming the alkaline solution of arsphenamine led in every case to a diminution in hemolytic power as compared with the degree of hemolysis produced by a given specimen kept at 0°C.   The specimens which Hunt (5) described

TABLE 2

DATE	SPECIMEN NUMBER	TOXICITY	TEMPERATURE	INCUBATED	HEMOLYSIS REACTION				
					Disodium arsphenamine 1 per cent				
					0.3 cc.	0.4 cc.	0.5 cc.	0.6 cc.	0.7 cc.
4/20/21	218	100	Kept at 0°C.	Yes	0	+	+++	++++	++++
				No	0	++	++	+++	+++
4/20/21	218	100	Stood at room temperature for 1 hour in alkaline solution	Yes	0	0	+	++	+++
				No	0	0	0	+	++
4/20/21	218	100	Warmed to 55° C. for 1 hour in alkaline solution	Yes	0	0	0	0	+
				No	0	0	0	0	0
6/22/21	139	60	Kept at 0°C.	Yes	+	++	+++	++++	++++
6/22/21	139	60	Warmed to 55°C. in acid solution for 1 hour	Yes	0	++	+++	++++	++++
6/22/21	147	120	Kept at 0°C.	Yes	0	+	++	+++	++++
6/22/21	147	120	Warmed to 55°C. in acid solution for 1 hour	Yes	0	0	++	+++	++++

as showing a diminution of toxicity on warming, manifested diminution of hemolytic power most of all.   These experiments were carried out by dissolving the hydrochloride in an ice-bath and adding the required amount of alkali at this temperature. The resulting solution was divided into three portions: one of which remained at 0°C., one stood at room temperature, and

one was warmed to 55°C. for one hour. The last two were immediately returned to the ice-bath at the expiration of an hour. Then the usual technique was followed. It was found that incubation at 37°C. did not affect the result as compared with putting the specimens immediately into the ice-box after adding the red cells and salt solution (cf. table 2). It was also found that warming solutions of the hydrochloride had only a very slight effect on the hemolytic power, when tested after being changed to the disodium salt.

In searching for the cause of the hemolytic power of arsphenamine we examined a number of substituted phenyl arsonic acids in the form of their sodium salts by the technique previously described. The following substances were tested in 1 per cent solutions in 0.82 per cent salt solution:

> o-hydroxy phenyl arsonate (sodium)
> 3′ 5-dinitro-4, hydroxy phenyl arsonate
> m-nitro, p-hydroxy phenyl arsonate
> p-hydroxy phenyl arsonate
> m-nitro, p-methyoxy phenyl arsonate
> p-methoxy phenyl arsonate
> p, p′-dihydroxy diphenyl arsonate
> p, o′-dihydroxy diphenyl arsonate
> p-amino phenyl arsonate
> 3, nitro 4, amino phenyl arsonate
> Sodium arsenite
> Sodium arsenate

All these were found to have no hemolytic power according to this method.

In addition, the following two polyarsenides were tested:

A. The polyarsenide of 4, 4′ dihydroxy-3, 5′ diamino arsenobenzene.
B. The polyarsenide of 2, 4, 4′ trihydroxy-5, 3′ diamino arsenobenzene.

When freshly dissolved they give a red solution which produces no hemolysis. They are precipitated during the test as shown by a clear yellow supernatant fluid in each tube. After standing several days in the ice-box, specimen B, however, was hemolytic (cf. table 3). The explanation of this reaction is not clear.

In an effort to account for the variations in hemolytic power of various samples of arsphenamine, it was thought necessary

TABLE 3

Hemolysis reaction

DATE	COMPOUND	TOXICITY	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.10	REMARKS
5/17/21	Polyarsenide A	140	—	—	0	0	0	0	0	—	—	—	Dissolved at 0°C. and kept at 0°C. Hypophosphorous acid preparation
4/16/21	Polyarsenide B	50–60	—	—	0	+	+++	+++	++++	—	—	—	Several days in icebox. Hyposulfite reduction
6/1/21	Dihydroxyarsenobenzene	35	—	—	—	0	0	0	0	0	—	—	
5/27/21	Monoaminodihydroxyarsenobenzene	120	—	—	0	0	0	+	+	—	—	—	
4/20/21	Diaminodihydroxyarsenobenzene	100	—	—	0	+	+++	++++	++++	—	—	—	Average specimen
5/24/21	Triaminodihydroxyarsenobenzene	80	—	—	++++	++++	++++	++++	++++	—	—	—	Quite stable. Note the effect of warming (table 4)
5/25/21	Tetraminodihydroxyarsenobenzene	60	0	+	+++	++++	++++	++++	—	—	—	—	Decomposed very rapidly. Note effect of warming (table 4)
5/21/21	Dinitrohydroxyarsenobenzene	25	0	0	0	0	0	0	0	0	0	0	Made up at room temperature in alkaline solution

to determine which group in the molecule conferred this power upon it. In view of the work on explosives done during the war, the effect of varying the number of amino groups in the molecule was investigated. This was found to explain the hemolytic powers of this type of compound (cf. table 3). It is apparent from our results with these compounds that the hemolytic activity increases in direct proportion to the number of amino groups introduced. Thus it will be seen that the dihydroxy-arseno-benzene is quite non-hemolytic whereas the tetramino-dihydroxy-arsenobenzene is the most hemolytic of the series. In the table it appears as though the triamino compound is more hemolytic than the tetramino; this is due to the fact that, with the increase in the number of amino groups oxidation proceeds more rapidly; the tetramino compound oxidizes very rapidly even during the simple manipulations described. The rapidity of the change in the tetramino compound is brought out by a study of the effect of temperature (cf. table 4) on the hemolytic activity of these substances. It will be seen that the activity of the tetramino compound is more reduced by warming than that of the other compounds. This is due presumably to its instability. We tested the hemolytic activity of the dihydroxydinitroarsenobenzene and found it to possess no hemolytic activity (cf. table 3). Since this compound is highly explosive, it can not be handled in the dry state; our concentrations, were, therefore, only approximate, though the solution used was a little over one per cent rather than under as determined by subsequent analyses by Mr. W. G. Christiansen.

The use of arsphenamine in anemia, especially primary anemia, suggested that it might be interesting to test its antihemolytic action in comparison with the antihemolytic action of sodium arsenite and arsenate as reported by Gunn, (6). · If washed red cells (sheep) be treated with equal quantities of sodium arsenate or arsphenamine (1:5000) for one hour, such cells acquire a resistance to immediate hemolysis by distilled water or hypotonic salt solution. While this treatment delays hemolysis, it will take place in the usual manner if the cells remain in contact with the salt solution or distilled water over night after incuba-

tion for an hour as in the experiments previously described. If however, cells so treated be used in the titration of complement[5] no delay in hemolysis by rabbit hemolysin can be demonstrated.

Disodium arsphenamine dissolved in physiological salt solution is no more hemolytic than when dissolved in 0.68 per cent salt solution when tested by the method described. The hemoly-

TABLE 4

COMPOUND	TEMPERATURE	HEMOLYSIS REACTION					
		0.2	0.3	0.4	0.5	0.6	0.7
Monoaminodihydroxyarsenobenzene	0°C.	—	0	0	++	++	+++
	Room temperature, one hour	—	0	0	0	++	+++
	55°C., one hour	—	0	0	0	0	+
Diaminodihydroxyarsenobenzene	0°C.	—	0	+	+++	++++	++++
	Room temperature one hour	—	0	0	+	++	+++
	55°C. one hour.	—	0	0	0	0	+
Triaminodihydroxyarsenobenzene	0°C.	—	++++	++++	++++	++++	++++
	Room temperature, one hour	—	0	++++	++++	++++	++++
	55°C., one hour	—	0	0	++++	++++	++++
Tetraminodihydroxyarsenobenzene	0°C.	+	+++	++++	++++	++++	—
	Room temperature, one hour	—	+	+++	++++	++++	++++
	55°C., one hour	—	0	+	++	+++	++++

sis here reported was completely inhibited if the red cells were suspended in serum instead of salt solution. This inhibiting effect of serum has been found to hold for many hemolytic agents (7). From these experiments it seems unlikely that any precautions (other than dissolving it in normal salt solution)

[5] These experiments were carried out by Dr. W. A. Hinton in the Massachusetts State Wassermann Laboratory.

against hemolysis by arsphenamine in its therapeutic administration are necessary.

It has been demonstrated that the hemolytic properties of arsphenamine are conferred upon it by the presence of the amino group. The importance of the conditions under which the nitro group is reduced in determining the modifications of toxicity of samples of arsphenamine varying but slightly in their preparation has been well emphasized in the studies of Christiansen (8). In a general way, slow reduction of the nitro group increases the hemolytic activity. The introduction of two nitro groups into dihydroxy-arseno-benzene does not cause the appearance of hemolytic activity. This touches the controversies over the relative effect of these groups in explosives. In the latter substances either the nitro or the amino groups may confer hemolytic properties on the resulting compound. Evidence similar to that brought out in relation to arsphenamine is afforded by the investigations of v. Braun and Rawicz (9).

These observers studied the effect of various changes in the o-amino-p-nitrotoluene, finding that this compound is non-hemolytic whereas toluenediamine is hemolytic. Lengthening the side chain has insignificant quantitative effects on this molecule but the joining of two such molecules by their side chains destroys the hemolytic activity. This compound behaves in an analogous manner to the dinitro and diamino arsenobenzenes. Apparently, therefore, in any chemotherapeutic studies involving the substitution of amino groups, hemolytic properties in their resulting compound must be considered.

SUMMARY AND CONCLUSIONS

1. A method is described which was used in testing the hemolytic activity of various samples of arsphenamine and allied substances using sheep's red corpuscles as a test object.

2. The hemolytic activity of various samples of arsphenamine was found to vary in a general way as did the toxicity, when the latter depended upon variations in the conditions of reduction of the nitro to the amino group in the preparation of the sample.

3. On standing or after shaking in alkaline solution, the hemolytic properties of a given sample decrease, often disappearing altogether.

4. "Arsenoxide" is non-hemolytic.

5. The sodium salts of various substituted phenylarsonic acids related to arsphenamine are non-hemolytic.

6. Warming a sample of disodium arsphenamine to 55°C. decreases its hemolytic power. Warming the hydrochloride causes comparatively little diminution of hemolytic power when tested after being changed to the disodium salt.

7. The hemolytic power of dihydroxyarsenobenzene (in 1 per cent solution as sodium salt) is nil, but the introduction of amino groups causes the resulting compounds to acquire hemolytic properties in direct proportion to the number of amino groups introduced.

8. The antihemolytic action of arsphenamine is similar to that described for sodium arsenate and arsenite when tested against chemical hemolytic agents; none of these substances exert an antihemolytic action against rabbit hemolysin.

9. The presence of serum inhibits hemolysis by arsphenamine.

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FEEDING EXPERIMENTS ON TADPOLES: PROSTATE GLAND AND OTHER SUBSTANCES

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The interesting observations of Gudernatsch (1) on the effects of feeding tadpoles with various glandular substances have been followed up by numerous investigators. His results on thyroid feeding have been confirmed and extended by the studies made in this laboratory (2–6). It has been shown that the *specific* effect of thyroid, on tadpoles (metabolic changes, evidenced by retarded growth and hastened metamorphosis), is caused by the iodine of the thyroid which is held in the colloid material of the gland (4), since glands that are markedly hyperplastic do not cause the same reaction. When thyroid gland is hydrolysed according to the procedure employed by Kendall (5), the product "A" represents the active iodine compound in concentrated form. Product "B" has a comparatively low iodine content, and when fed to tadpoles "A" causes a strikingly greater effect than the gland from which it was obtained while "B" is inactive or causes only such an effect as might be due to the presence of minute quantities of "A" owing to incomplete separation. The specificity of this reaction enables its use as a biological method for assaying the physiological value of commercial thyroid preparations (6). Abderhalden (7) studied the effect of feeding tadpoles with various endocrine glands. He used the glands separately, in combination with each other and in fractions obtained by hydrolysis, and added further evidence of the well known different effects caused by thyroid and thymus glands. He also obtained results similar to those of Rogoff and Marine

(5), with thyroid products obtained by hydrolysis of the gland. Abderhalden interprets his observations as showing a definite specific influence on the growth and development of tadpoles when fed with pituitary gland, ovary, testes, and adrenal gland.

Macht (8) observed increased growth and hastened metamorphosis of tadpoles when fed with prostate gland. Through the courtesy of Dr. Macht some of the tadpoles that he had fed with prostate and also their controls were seen by one of us (J. M. R.) while in Baltimore in 1919. The specimens observed showed the differences between the controls and the prostate-fed tadpoles illustrated in Macht's paper, and his results led us to repeat the work with the idea of extending it if possible.

The results of our experiments failed to convince us that they may be interpreted as showing a specific effect on growth or development of frog larvae caused by feeding prostate gland. Certainly no effect was seen that is comparable to that caused by small doses of thyroid containing only small amounts of colloid and iodine. Similar effects to those produced by prostate feeding were seen in tadpoles receiving other substances used as controls.

With the tadpole reaction, as with all other biological tests, there are sources of error which must be carefully eliminated, if possible, or else very carefully controlled. Failure to eliminate reactions due to conditions other than those experimentally introduced may easily lead to misinterpretation of results. We have frequently seen a number of the stock tadpoles, which were not being used for the experiment, to undergo more rapid development than the others, even when they were kept in the refrigerator. This phenomenon is sometimes associated with retardation of growth and again it may be seen with more rapid growth than the rest of the tadpoles in the basin. It is sometimes seen, in some of the sets of a series of feeding experiments, that one or two tadpoles out of ten or twelve show this peculiarity. These must, of course, be excluded to avoid possible misinterpretation of the results.

We carried out two series of observations using the larvae of Rana pipiens and Rana palustris. Twenty-three different

specimens of human prostate[1] and two commercial preparations (ram's prostate, and glands from bullocks) were fed to the tadpoles. Of the human material six specimens were obtained at autopsy from patients who died from various causes. One was from a young man twenty years old and the fresh gland weighed 10 grams, another was from a man thirty-four years old, the fresh gland weighing 25 grams. The other four specimens were from older men (44, 57, 62, and 75 years old) and weighed respectively 51, 44, 32 and 31 grams. The rest of the human material was obtained in the operating room, following prostatectomies, from men ranging in age from 53 to 88 years, the weights of the fresh glands ranging from 12 to 278 grams. Histologically, most of the glands obtained at operation showed chronic prostatitis with hyperplasia. Three glands showed adenoma and one adeno-carcinoma. Since some of the specimens were normal glands from young individuals, while the others represented various grades of abnormal glands, the conditions were favorable for the detection of differences in effect on the tadpoles if they existed. As controls fresh liver was given for one hour each day in one set and every other day in another set, in both series. Further controls were made by feeding with fresh liver, boiled liver, lymph glands, cattle blood-serum, cracker dust, and an old specimen of brewer's yeast, all of these being desicated.

In the first series (May 18-June 9) the tadpoles were fed with the prostate and control substances in 50 mgm. doses every other day and fresh liver for one hour on the alternating days. In the second series (June 9–25) the same amount of material was fed daily, with fresh liver offered for half the usual period every other day. Feeding was continued until most of the tadpoles showed legs or well marked leg buds. This was seen, in both series, in the tadpoles getting prostate or the other substances several days before it was manifested in those getting only fresh liver daily. At the time when the experiment was discontinued the tadpoles receiving fresh liver only on alternating days did not yet show leg buds and were smaller than any of the others.

[1] We are indebted to Drs. W. E. Lower and P. A. Jacobs for the human material.

The tadpoles receiving fresh liver every day grew more rapidly than those getting liver every other day and the tadpoles getting the desiccated products grew more rapidly than either.

It appears, therefore, that some increase of growth and development can be induced by feeding tadpoles with many substances, in addition to liver used as control, and it must be pointed out that if only the tadpoles receiving fresh liver were used as controls it would be possible to interpret the results obtained by us with the prostate glands as positive. But this only emphases the importance of making adequate controls in experiments of this nature. For it is this disturbing factor that must be eliminated in feeding experiments with thyroid substance when the product is very weak in active material, and only such results should be interpreted as positive as clearly show a more definite effect than the above mentioned phenomena, which may be caused by many substances other than thyroid. Since thyroid retards growth while the other materials studied seem to increase growth the difficulty is not so great as might be apparent.

That many substances might have a perceptible influence on growth and development when fed to frog larvae is not surprising, for the food offered and the other conditions of the experiment are very different from the normal for tadpoles. This does not, however, affect the usefulness of the reaction for thyroid if, as should be the case with all biological tests, the disturbing factors are, as far as possible, eliminated or controlled.

A number of human prostates and one commercial preparation (ram) showed evidence of metamorphosis five or six days before any of the others. One specimen caused death of all the tadpoles in one day when fed in 50 mgm. doses but was about equal in effect to those above mentioned when the dose was reduced to 15 mgms. On looking into the matter it was found that this specimen had been preserved in formalin for a few days before being desiccated. Two of the others had been similarly treated but were in the preservative for only a short while (a few minutes). Two or three specimens had not been placed into the drying oven soon enough to avoid some putrefaction. These specimens gave effects which could easily have

been interpreted as positive if the series were not a large one and the effects caused by these specimen eliminated by numerous controls.

It is possible that by decomposition into simpler substances through autolysis, hydrolysis or other means most animal tissues might yield certain products capable of affecting growth and development of tadpoles. This appears to be the basis of Abderhalden's work. But we are led to believe, from our experiences up to the present time, that a *specific* influence is demonstrable only by feeding with thyroid containing colloid and iodine.

Chemical examinations for iodine were made on a number of the specimens (when sufficient amount was available) and on the two commercial preparations of prostate, with negative results.

On the basis of our experience with the tadpole reaction we are led to conclude that, of the various endocrine glands heretofore studied, only thyroid substance (containing colloid and iodine) when fed to frog larvae causes specific effects on their growth and metamorphosis. Dr. Macht (9) states "that on feeding various glandular tissues to tadpoles, I found that the prostate simulated to some extent the effect of the thyroid, but was very much weaker. As to this effect being a specific property of a prostatic hormone, the question is still entirely unsettled."

Our experience with prostate and the results obtained by Macht as well as the observations of others indicate that some changes can be caused when tadpoles are fed with various endocrine glands. But we share with Dr. Macht the opinion that this effect does not indicate a specific hormone action. The effects observed on feeding tadpoles with these substances are not great or constant enough to impair the usefulness of the reaction as a test for thyroid substance.

SUMMARY

Feeding experiments with tadpoles indicate that a number of substances may increase growth and sometimes slightly hasten development. This is, however, not comparable with the *specific* effect on growth and metamorphosis caused by thyroid feeding.

Of twenty-three human prostates (normal and diseased) and two commercial preparations (ram and bullock) none gave evidence which could be interpreted as a specific effect on the growth or metamorphosis of frog larvae.

The effects produced by lymph gland, boiled liver, fresh liver, cracker dust, brewer's yeast, (desiccated) illustrates the disturbing factors in this biological test for thyroid which must be eliminated, as far as possible, by control observations.

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ANIMAL CALORIMETRY

Twenty-First Paper

THE INFLUENCE OF MORPHINE UPON HEAT PRODUCTION IN THE DOG

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I. INTRODUCTION

Ordinary observation of the action of morphine on the organism would naturally lead to the conclusion that the metabolism is lowered, since there is a lessened rate of respiration, slower pulse and a lower body temperature. The evidence at hand, however, is inconclusive.

Von Boeck (1) in 1871 determined the influence of morphine on the protein metabolism of a dog in nitrogen equilibrium and concluded that it was reduced by 6 per cent. Underhill, Goldschmidt and Blatherwick (2), however, found a definite increase in the urinary nitrogen output after injecting morphine in a starving dog.

Bloor (3) found that the fat content of the blood was unchanged during morphine narcosis but that a lipemia resulted two days later.

Luzzatto (4) and Araki (5) first demonstrated the presence of a reducing sugar in the urine of rabbits and dogs after large doses of morphine. A study of the blood-sugar content was made by Ross (6) after injections of morphine (10 mgm. per kilogram body weight). The analyses were continued until one and a half hours after the injections, but no results are given to

359

indicate when the normal blood-sugar level was restored. A maximum increase of 77 per cent in the blood-sugar content was obtained one and one-half hours after the injection of morphine.

The output of carbon dioxide and the consumption of oxygen in cats and dogs after administering morphine were studied by von Boeck and Bauer (7) as early as 1874. They found that there was an increased respiratory exchange in the excited cat but that in the narcotized dog the carbon dioxide output was reduced 27 per cent and the oxygen consumption, 34 per cent. Voit (8) concludes from these results that morphine affects metabolism only indirectly because of decreased muscular activity; that in narcosis, as in sleep, there is a reduction in the production of heat. Normal control animals were not at that time restricted in their movements during the measurement of their metabolism.

The oxygen consumption of two human beings in normal and morphine sleep was studied by Loewy (9) and in both cases there was found to be a slight decrease of oxygen utilization after morphine administration. He concludes that morphine does not materially affect the respiratory exchange. Higgins and Means (10) obtained diabetic quotients on themselves after therapeutic doses of morphine. The experiment was continued for two hours after administration of the drug. The respiratory exchange showed no alteration in the consumption of oxygen, but there was a marked drop in carbon dioxide elimination.

The alkali reserve of the blood plasma is either unaltered or slightly depressed during sleep (Collip (11)). Hjort and Taylor (12) and Gauss (13) found an increased alkali reserve in narcotized dogs after morphine.

The purpose of this investigation is to study the effects of subcutaneous injection of morphine upon the basal metabolism of dogs.

II. METHODS

Two trained calorimeter dogs, which had received the "standard diet" at 5 p.m. the day previously, were used in these experiments. Doses varying from 12 mgm. to 20 mgm. of mor-

phine (in 2 per cent solution) per kilogram body weight were injected subcutaneously, and the dog was placed in the calorimeter immediately after vomiting and defecation had ceased. The urine for the period was collected by catheterization.

The basal metabolism was determined at frequent intervals about eighteen hours after administration of a standard diet when the dog was resting quietly in the calorimeter at a temperature of 26°C. It is important to note that the trained dog remains quiet or asleep throughout the experiment.

III. BASAL METABOLISM

The determinations of the basal metabolisms of the two dogs may be summarized as shown in table 1.

TABLE 1

Basal metabolisms of dogs XVIII and XIX

NUMBER OF EXPERIMENT	DATE	HOURS OF EXPERIMENT	R. Q.	CALORIES	
				Indirect	Direct
Dog XVIII					
	1921				
76A	February 11	2	0.82	32.36	30.45
77	February 12	2	0.75	32.24	30.83
78	February 17	2	0.82	34.31	37.47
80A	February 25	1	0.83	17.12	17.04
Sum......................		7		116.03	115.79
Average per hour......................			0.81	16.58	16.54
Dog XIX					
55A	February 4	2	0.79	34.58	32.71
57	February 15	1	0.82	17.47	18.09
59	February 23	2	0.85	35.38	36.45
Sum......................		5		87.43	87.25
Average per hour......................			....0.82	17.49	17.45

These results may be compared with former determinations upon these dogs as follows:

	DOG XVIII	DOG XIX
Date.....................................	Exp. 57, May 9, 1919	Jan. to Apr., 1920
Weight in kilograms......................	10.8	10.6 to 9.6
Calories of metabolism...................	16.6	17.61
Present series (1921):		
Weight in kilograms..................	11.05 to 10.65	9.65 to 9.15
Calories of metabolism...............	16.56	17.49

Two years of continuous confinement in a metabolism cage did not change the basal metabolism of dog XVIII.

IV. ADMINISTRATION OF MORPHINE

The two dogs behaved differently after administering morphine. Dog XVIII was usually absolutely quiet throughout the period

TABLE 2

Influence of morphine upon the basal metabolisms of dogs XVIII and XIX

NUMBER OF EXPERIMENT	DATE	HOURS OF EXPERIMENT	MORPHINE PER KILOGRAM	R. Q.	CALORIES	
					Indirect	Direct
Dog XVIII						
	1921		*mgm.*			
76B	February 11	3	15	0.79	47.29	40.73
79	February 18	4	15	0.85	62.17	61.91
80B	February 25	3	15	0.84	44.81	?
81	March 1	3	15	0.78	47.91	47.86
Sum....................		13			202.18	
Average per hour...........................				0.82	15.55	
Dog XIX						
55B	February 4	2	12	0.90	35.23	33.55
58	February 16	3	20	0.87	61.18	54.83
60	February 24	2	15*	0.84	43.00	42.67
61	February 28	4	15†	0.82	74.90	74.50
Sum....................		11			214.31	205.55
Average per hour...........................				0.86	19.48	18.69

*With ether and chloroform.
†With ether.

of experimentation, whereas dog XIX showed increased irritability, with heightened reflexes. An attempt was made to more heavily anesthetize this animal by giving ether or ether and chloroform but without entire success.

The results obtained may be summarized as shown in table 2.

These results show that in dog XVIII the administration of morphine reduced the basal metabolism from 16.56 to 15.54 calories per hour, an average fall of 6.2 per cent; whereas in dog XIX the metabolism was increased from 17.49 to 19.48 calories, a rise of 10 per cent.

If one considers the heat production in the first experimental hours in the morphine experiments upon dog XVIII one finds an average of 14.7 calories per hour. This represents an average reduction of 11 per cent in the basal metabolism of this dog. The metabolism usually returned to the normal basal level in the third and fourth hours, even though the body temperature was lowest during these hours, as may be seen below:

DOG XVIII	EXPERIMENT 76B	EXPERIMENT 79	EXPERIMENT 80B	EXPERIMENT 81	AVERAGE REDUCTION BELOW THE BASAL VALUE
					per cent
2nd hour.......	14.44	14.73	13.71	15.80	11
3rd hour	16.42*	15.22	15.78	15.08	6
4th hour	16.42*	15.38	15.32	17.03	4
5th hour		16.84			

* Not separately determined.

In the excitable dog XIX no analogous diminution of metabolism was noted. The highest metabolism, at the rate of 21.5 calories per hour, was observed when morphine, chloroform and ether were administered together. This is an increase of 4 calories, or 23 per cent, above the basal metabolism. As the activity of the dog was less than when morphine and ether were given together one may surmise a calorigenetic action on the part of chloroform. The usual fall in body temperature did not occur in this experiment, so this factor as an element in reducing metabolism was not in evidence.

The respiratory quotients in dog XIX rose somewhat after the administration of morphine. This may be due to the well-known preference of active muscle for carbohydrate. In dog XVIII, at least, the great increase in blood-sugar after giving morphine, which was reported by Ross, apparently had little effect qualitatively or quantitatively upon the metabolism of the animal.

V. THE MANNER OF HEAT LOSS

During the determination of the basal metabolism it is often noted that the rectal temperature of the quiet dog falls a few tenths of a degree. Such a fall denotes a loss of the body's heat to the external air by radiation and conduction. After the administration of morphine the body temperature of the dogs fell between 0.95 and 2.05°C. during the experimental periods, except in the one instance already mentioned when ether and chloroform were also administered.

In experiment 77, in which a determination of the basal metabolism was made on dog XVIII, 23.82 (72 per cent) calories were eliminated by radiation and conduction and 9.21 (28 per cent) by vaporization of water from the lungs, a total of 33.03 calories in two hours. The calculated metabolism of the period was 32.24 calories. The dog's body lost in temperature 0.24°C., as measured by the rectal thermometer, a loss which is the equivalent of 2.2 calories eliminated from his body. [11 (kgm. body weight) × 0.83 (specific heat of the body) × 0.24 (fall in body temperature) = 2.2 calories eliminated.] Deducting this from the 33.03 calories "eliminated," one obtains 30.83 calories as calories "produced," which are the calories directly measured. In long experiments, in which the changes in the body temperature play a negligible part in the results obtained, the calculated calories (indirect calorimetry) correspond with the calories produced (direct calorimetry). The body temperature is best measured by the rectal thermoneter, but there are evidently indeterminable factors here which render exactness impossible. If one assumes that the above calculation by indirect calorimetry gives the more correct value of the actual amount of heat pro-

duced, then one may state that of the 32.24 calories which were produced 33.03 were eliminated; also, that when 100 were produced 74 were lost by radiation and conduction, 29 by the evaporation of water, a total of 103. Three per cent more calories (0.79 calorie) were therefore eliminated than were produced.

Understanding these principles, one may analyze from the accompanying table 3 the profound losses of heat from the dog's body after giving morphine. For example, in experiment 81 on dog XVIII the rectal thermometer indicates a cooling of the dog's tissues which corresponds to a loss of 18.12 calories during three hours. Indirect calorimetry indicates a production of heat amounting to 47.91 calories, while the heat loss measured by the calorimeter was 65.98 calories, or 18.07 calories more than were produced, as estimated by indirect calorimetry. The results check perfectly. Thirty-six per cent more calories were lost from the body than were produced. More heat was lost by radiation and conduction alone (106 per cent) than was produced during the interval. All this indicates a distribution of heat to the area of the skin by relaxed blood vessels. The demonstrated increased loss of heat by radiation and conduction from the skin recalls the dilation of the superficial blood vessels in man after the administration of Dovers powder, which contains morphine and ipecac. Though the heat eliminated per hour is 22 calories, or 32 per cent above the basal level, the heat production is 16 calories, or only 4 per cent below that level. The reduction of metabolism by approximately half a calorie an hour cannot possibly be the cause of a loss of 6 calories per hour with the consequent fall in body temperature. The relative methods of heat loss are not materially changed from the normal, for 22 per cent of the calories were eliminated by vaporization of water, 78 per cent by radiation and conduction. The average of the four normal periods shows a heat loss of 26 per cent by the vaporization of water.

In experiment 80B the average heat loss from the body is 4.6 calories per hour above the heat production as calculated by the indirect method, whereas this heat production is only 1.6 calories below the basal level.

TABLE 3

Influences of morphine upon the manner of heat loss; values in calories per hour

	DOG XVIII				DOG XIX			
	Experiment 76B	Experiment 79	Experiment 80B	Experiment 81	Experiment 55B	Experiment 58	Experiment 60 plus chloroform and ether	Experiment 61 plus ether
	calories	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*
Basal metabolism.................	16.56	16.56	16.56	16.56	17.49	17.49	17.49	17.49
Indirect calorimetry after morphine...........	*15.76*	*15.54*	*14.94*	*15.97*	*17.61*	*20.39*	*21.50*	*20.51*
Change from basal................	−0.80	−1.02	−1.62	−0.59	+0.12	+2.90	+4.01	+3.02
Calories eliminated*...........	18.65	18.42	19.56	22.00	20.46	22.43	21.50	20.51
Indirect calorimetry............	*15.76*	*15.54*	*14.94*	*15.97*	*17.61*	*20.39*	*21.50*	*18.72*
Loss of calories from body..........	−2.89	−2.88	−4.62	−6.03	−2.85	−2.04	±0	−1.79
Same (calculated from rectal temperature).......	−5.08	−2.85		−6.04	−3.46	−4.15	−0.32	−3.84
Per cent of calories eliminated in water vapor.......	25	20	18	23	21	21	21	26

* By radiation and conduction and by vaporization of water. The latter factor in dog XVIII was 26, in dog XIX 23 per cent of the total in the average of 4 and 3 determinations of the basal metabolism.

Perhaps even more striking are the results upon the irritable dog XIX. Calculated by the same method there is an hourly excess of heat loss of 2 calories from the animal's body in experiment 58, despite an increase in the heat production of 2.90 calories above the basal level. The heat loss by vaporization of water was 21 per cent, in contrast with 23 per cent as the average of three determinations of the basal metabolism.

The results of all the experiments in which morphine was given may be briefly condensed into table 3.

Consideration of this table shows that, except in the case in which chloroform was also administered, there was in every instance following the administration of morphine a loss of heat from the body which was not compensated for by a corresponding increase in metabolism, as is normally the case. This loss of heat occurred in a quiet resting dog (dog XVIII) despite the fact that the decrease in heat production was relatively slight; and it occurred also in the dog with heightened reflexes despite the increase in metabolism.

VI. ALCOHOL CHECKS

The validity of the work here presented is supported by the following alcohol checks. The calculated heat production and the theoretical oxygen consumption were estimated from the carbon dioxide production of the burning alcohol.

TABLE 4

Average of alcohol checks

NUMBER OF EXPERIMENT	DATE	R. Q.	PER CENT ERROR ON O_2	CALORIES	
				Indirect	Direct
	1921				
84	January 24	0.671	−0.6	68.20	67.98
85	February 3	0.651	+2.4	68.50	67.04
86	February 8	0.650	+2.6	65.05	62.19
88	February 10	0.667	±0	43.32	41.45
89	March 2	0.658	+1.3	70.83	69.41
90	March 3	0.676	−1.3	34.54	38.21
		0.662	+0.7	350.44	346.28

TABLE 5

Dog XVIII

DATE	EXPERIMENT NUMBER	TIME	CO₂	O₂	R.Q.	H₂O	Urine N	CALORIES				BODY TEMPERATURE		MORNING WEIGHT	BEHAVIOR OF DOG	FOOD
								Protein	Non-protein	Indirect	Direct	Start	End			
1921			*gm.*	*gm.*		*gm.*	*gm.*							*kgm.*		
Feb. 11	76A	10.50–12.50	10.94	9.69	0.82	15.13	0.201	5.34	27.02	32.36	30.45	37.41	37.11	11.05	Quiet	Basal metabolism
Feb. 11	76B	2.15– 3.15	4.84	4.35	0.81	8.36	0.118	3.15	11.29	14.44	12.57	36.57		11.05	Quiet	Morphine, 15 mgm. per kilogram, 2 injections at 1 and 1.40 p.m., 35 minutes before start
		3.15– 5.15	10.69	9.96	0.77	15.35	0.236	6.30	26.55	32.85	28.16		34.91			
										47.29	40.73					
Feb. 12	77	10.35–11.35	5.06	4.96	0.74	8.23	0.092	2.44	13.79	16.24	14.52	36.53		11.05	Quiet	Basal metabolism
		11.35–12.35	5.06	4.87	0.76	7.50	0.092	2.44	13.56	16.00	16.31		36.29		Quiet	
										32.24	30.83					
Feb. 17	78	2.18– 3.18	5.68	4.87	0.85	8.79	0.085	2.25	14.15	16.40	18.21	38.13		10.85	Quiet	Basal metabolism
		3.18– 4.18	5.84	5.40	0.79	7.77	0.085	2.25	15.66	17.91	19.28		37.65			
										34.31	37.49					
Feb. 18	79	11.09–12.09	5.37	4.36	0.90	6.37	0.143	3.79	10.94	14.73	13.65	36.25		10.80		Morphine, 15 mgm. per kilogram, 2 injections at 9.31 and 10.10 a.m., 1 hour before start
		12.09– 1.09	5.43	4.53	0.87	6.61	0.143	3.79	11.43	15.22	16.10					
		1.09– 2.09	5.43	4.59	0.86	6.37	0.143	3.79	11.59	15.38	15.86					
		2.09– 3.09	5.54	5.11	0.79	6.33	0.143	3.79	13.05	16.84	16.30		34.98			
										62.17	61.91					

Feb. 25	80A	11.32–12.32	5.82	5.12	0.83	5.87	0.104	2.76	14.36	17.12	17.04	37.45	37.36	10.65		Basal metabolism
Feb. 25	80B	1.56– 2.56	5.20	4.02	0.94	5.86	0.144	3.82	9.89	13.71					Quiet	Morphine, 15 mgm. per kilogram, 30 minutes before start. Rectal thermometer broken
		2.56– 3.56	5.29	4.77	0.81	6.24	0.144	3.82	11.96	15.78						
		3.56– 4.56	5.06	4.65	0.79	6.33	0.144	3.82	11.50	15.32						
										44.81					10.65		
Mar. 1	81	2.05– 3.05	5.01	4.85	0.75	9.18	0.154	4.08	11.72	15.80	17.30	37.46		10.50	Quiet	Morphine, 15 mgm. per kilogram, 45 minutes before the start
		3.05– 4.05	5.19	4.55	0.83	8.41	0.154	4.08	11.00	15.08	15.29					
		4.05– 5.05	5.40	5.22	0.75	7.99	0.154	4.08	12.95	17.03	15.27		35.41				
										47.91	47.86						

TABLE 6
Dog XIX

DATE	EXPERIMENT NUMBER	TIME	CO₂	O₂	R.Q.	H₂O	Urine N	CALORIES				BODY TEMPERATURE		MORNING WEIGHT	BEHAVIOR OF DOG	FOOD
								Protein	Non-protein	Indirect	Direct	Start	End			
1921			gm.	gm.		gm.	gm.							kgm.		
Feb. 4	55A	10.45–11.45	5.58	5.17	0.78	5.78	0.092	2.44	14.69	17.13	15.96	37.95		9.35	Quiet	Basal metabolism
		11.45–12.45	5.70	5.26	0.79	5.78	0.092	2.44	15.17	17.45	16.75		37.79		Quiet	
										34.58	32.71					
Feb. 4	55B	2.14– 3.14	6.17	4.75	0.94	7.24	0.092	2.44	13.92	16.36	17.05	36.40		9.35	Restless	Morphine, 12 mgm. per kilogram at 1 p.m., 1¼ hours before start
		3.14– 4.14	6.66	5.54	0.87	7.54	0.092	2.44	16.43	18.87	16.50		35.54		Restless	
										35.23	33.55					
Feb. 15	57	2.27– 3.27	5.87	5.23	0.82	6.18	0.096	2.54	14.93	17.47	18.09	38.34	38.40	9.15	Quiet	Basal metabolism
Feb. 16	58	10.55–11.55	6.61	5.67	0.85	8.09	0.122	3.23	15.82	19.05	17.55	37.55		9.20	Occasional movements	Morphine, 20 mgm. per kilogram given 50 minutes before start
		11.55–12.55	7.45	6.01	0.90	7.81		3.23	17.26	20.49	17.22				Moving 6 minutes	
		12.55– 1.55	7.66	6.39	0.87	7.97		3.23	18.41	21.64	20.06		35.92		Very restless	
										61.18	54.83					
Feb. 23	59	1.36– 2.36	6.39	5.23	0.89	7.76	0.162	4.29	13.37	17.66	18.84	38.09		9.65	Quiet	Basal metabolism
		2.36– 3.36	6.05	5.33	0.83	7.23			13.43	17.72	17.61		38.15		Quiet	
										35.38	36.45					

Date	No.	Time											Remarks	Treatment
Feb. 24	60	2.40- 3.40	7.43	6.34	0.85	7.78	0.133	3.53	17.81	21.34	20.80	36.50	9.55	Moving 4 minutes	Morphine, 15 mgm. per kilogram at 1.30 p.m. Ether 10 minutes, then chloroform until corneal reflex disappeared
		3.40- 4.40	7.43	6.46	0.84	7.72	0.133	3.53	18.13	21.66	21.87	36.46		Moving 3 minutes	
										43.00	42.67				
Feb. 28	61	12.02- 1.02	6.99	5.92	0.86	10.09	0.179	4.74	15.12	19.86	17.88	37.45	9.60	Moving 1 minute	Morphine, 15 mgm. per kilogram at 11 a.m. Ether 5 minutes until corneal reflex disappeared
		1.02- 2.02	6.44	5.49	0.85	8.25	0.179	4.74	13.62	18.36	17.90	36.05		Occasional movements	
		2.02- 3.02	6.09	5.48	0.81	8.73	0.179	4.74	13.38	18.12	17.36			Quiet	
		3.02- 4.02	6.16	5.63	0.80	9.14	0.179	4.74	13.82	18.56	17.38			Moving 1 minute every 10 minutes	
										74.90	70.52				

Although these alcohol checks are by no means perfect, it is evident that in general the results obtained by the calorimeter must have been trustworthy during the month of February when the experiments showing the influence of morphine upon the metabolism of dogs were accomplished.

VII. SUMMARY

1. Two dogs reacted differently to doses of between 12 and 20 mgm. of morphine per kilogram of body weight.

2. Dog XVIII, which remained absolutely quiet in morphine sleep, showed during the second hour after treatment an average fall in the heat production of 11 per cent below the basal level, for the third hour 6 per cent, for the fourth hour 4 per cent. The average reduction for all the periods was 6.2 per cent. The respiratory quotients were unchanged by administration of morphine.

3. In a typical experiment upon this dog the heat production was 0.6 calorie per hour less than the basal value of 16.6 calories, but the animal lost from its body to the calorimeter 6 calories per hour, or 32 per cent, more than it produced. The decrease in heat production cannot therefore be the cause of the fall in body temperature. There was no abnormal condition in the division of heat loss as between radiation and conduction and the evaporation of water.

4. Dog XIX manifested increased irritability with heightened reflexes after administering the drug. The basal metabolism increased by an average of 10 per cent. The maximum increase was 23 per cent after administering morphine, ether and chloroform. The respiratory quotients were higher after giving morphine to this dog, perhaps due to the preference of the active muscle for carbohydrate.

5. The increase in the metabolism of this irritable dog did not prevent a loss of body heat and consequent fall in body temperature after giving morphine. The pathways for the distribution of the heat loss were relatively unaltered.

6. In one experiment when morphine, ether and chloroform were given together there was no change in the body temperature.

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STUDIES OF CHRONIC INTOXICATIONS ON ALBINO RATS

VI. LEAD CARBONATE

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INTRODUCTION

Chronic lead poisoning is one of the best studied examples of chronic intoxications. Its symptomatology is well known, although far from being completely explained. It was formerly cited as a paradigm of cumulative poisoning, until Straub suggested and Erlenmeyer confirmed that the lead does not seem to accumulate in the body; so that the phenomena appear to be the result merely of the long-continued passage of lead through the body, requiring a certain dosage per day (i.e., a certain concentration), and a certain minimum time for their development. The older explanation considered the delay in the symptoms as equivalent to the time required to accumulate a sufficient store of lead; according to Straub's conception, it would correspond rather to the time required for the cumulation of the small injuries produced by single doses. By either theory, the delay would be shortened by higher doses; and by either theory, very small doses would be ineffective. According to the storage theory, they would become ineffective when they fell below the capacity of excretion; according to the Straub theory, when the single doses fell to the point where they ceased to injure the functions of the tissues.

The dosage required to produce "chronic lead poisoning" varies with a number of factors: The time of administration;

375

TABLE 1

Dosage for chronic lead poisoning; arranged in order of daily dosage

INVESTIGATOR	LEAD COMPOUND	ANIMAL	CHANNEL	MILLIGRAMS PER KILOGRAM PER DAY	DURATION OF ADMINISTRATION	TOTAL MILLIGRAMS PER KILOGRAM	EFFECT
Sollmann........	Carbonate	Rats	Food	0.00073	27 weeks	0.125	No effect
Lewin...........	Water	Homo	Oral	0.01–0.028			Said to be deleterious to health
Kobert.........		Homo	Oral	0.017			Chronic intoxication
Goadby and Legge.........		Homo	Oral	0.03	Years		Minimal effective dose
Gaertner.......		Homo	Oral	0.02–0.1	Several months	2–10	Symptoms
Erlenmeyer.....	Carbonate	Cats	Hypodermic	0.1	3–24 weeks	2–22	No effect
Sollmann.......	Carbonate	Rats	Food	0.12	19 weeks	16	No effect
Hayhurst.......	Carbonate	Homo	Oral	0.2	2 weeks	3	Symptoms
Brouardel.......	Water, 14 mgm. per liter	Homo	Oral	ca 0.25	?	?	Epidemic
	Water, 20 mgm. per liter	Homo	Oral	ca 0.3	?	?	Epidemic
Gaertner.......		Homo	Oral	1	3–4 weeks	20–30	Symptoms
Erlenmeyer.....	Carbonate	Cats	Hypodermic	0.8–1.4	9 weeks	43–75	Nervous symptoms and death in about 60 days
Straub.........	Sulphate or carbonate	Cat	Hypodermic	ca 1–1.5	9 weeks	ca 100	Severe nervous symptoms, death
Sollmann.......	Carbonate	Rats	Food	1.22	8 weeks	59	

Hanzlik	Shot	Duck	Crop	12	2 weeks	160	Dead in 2 to 2½ weeks
Hanzlik	Shot	Duck	Crop	14	2½ weeks	250	Dead in 2 to 2½ weeks
Weller	Carbonate	Guinea-pig	Capsules	14–30	15–50 weeks	2300–9400	Loss of weight
Hanzlik	Shot	Pigeons	Crop	23	3½ weeks	600	Dead in 3½ weeks
	Sulphate	Dog	Oral	40	1 day	40	No effect
Carlson and Woelfel Series I.	Carbonate	Dog	Oral	40	2 day	40	Considerable digestive disturbances, vomiting, etc., lasting about 6 days
Leymann	Sulphate	Cats	Oral	ca 80	8–9 weeks	ca 700	Toxic symptoms
	Carbonate	Dog		100	1–2 days	100–200	Severe digestive symptoms in 1 to 2 days
Carlson and Woelfel, Series II.	Sulphate	Dog		100	3–4 days	300–400	Mild digestive symptoms in 3 to 4 days
Carlson and Woelfel, Series V.	Carbonate	Cats	Capsules	100	2–8 days	200–800	Considerable digestive symptoms
	Sulphate	Cats	Capsules	100	2–8 days	200–800	Mild digestive symptoms
Goadby	Carbonate	Cats	Oral	200–300	4–72 weeks		Practically no symptoms

the absorbability of the compound; the criteria or degree of plumbism; the species of animal; and there are doubtless other conditions.

The relevant data that I have found in the literature are presented in table 1. In this I have also included the minimal, median and maximal doses of the present series of experiments.

These data show that daily doses of 0.01 to 0.03 mgm. per kilogram are said to produce poisoning in man, but with doubtful data. Absorption of 0.1 mgm. per kilogram per day from hypodermic injection does not poison cats, even in twenty-four weeks (Erlenmeyer).

Daily ingestion of 0.2 to 0.3 mgm. per kilogram appears to be the usual dosage in human lead epidemics from leaden waterpipes, where the dosage can be rather definitely calculated from the lead content of the water (12 to 20 mgm. of lead per liter; Brouardel).

Daily absorption of 1 to 1.5 mgm. per kilogram (from hypodermic deposits of lead carbonate, Erlenmeyer and Straub) is the smallest dosage that has been shown to produce definite symptoms in animals. The symptoms occur after a latent period of about five weeks, but rapidly become severe and terminate with death; i.e., they are much more severe than the symptoms of most clinical cases.

Daily dosage of 12 to 30 mgm. per kilogram is the smallest that has been reported as producing definite symptoms in animals, by oral administration. Guinea-pigs, to whom lead carbonate was administered by capsules (Weller), in daily dosage of 14 to 30 mgm. per kilogram for fifteen to fifty weeks, only lost in weight. Birds poisoned by shot placed in the crop, died with severe lead symptoms in two to three and one-half weeks, after having dissolved a daily average of 12 to 23 mgm. of lead per kilogram (Hanzlik).

A single oral dose of 40 mgm., of lead carbonate, per kilogram of dog, produces marked digestive disturbance, lasting about six days (Carlson and Woelfel).

To summarize: Phenomena of lead poisoning appear after some weeks with daily oral doses of lead salts, of 0.2 to 0.3 mgm.

per kilogram in man; and of 12 to 30 mgm. per kilogram in animals. The difference may be due partly to more accurate observation on men; partly to differences in response; and partly to differences in absorption. There is considerable difference in the degree of symptoms for a given dosage between birds and guinea pigs, and when actual absorption is measured (hypodermic deposits), cats are poisoned by 1 to 1.5 mgm. per kilogram.

DOSAGE AND DURATION OF THE EXPERIMENTS OF THIS SERIES

The present investigation was started with minimal doses (0.0005 to 1.5 mgm. of lead carbonate per kilogram of rat per day) continued over long periods of time (eight to thirty-five weeks), since the arrangements employed by us are especially suitable for revealing the minimal effects. Previous investigators (Brouardel, Erlenmeyer, Weller) have pointed out that loss of weight or check of growth is the first and most delicate index of chronic lead poisoning, and in fact the only symptom in the earlier stages. Our method is especially adapted to discover such check of growth.

It may be premised that the doses employed in this series very generally produced slight but definite loss of appetite and retarded growth; but no other symptoms.

The actual data are shown in table 2. The rats of experiment 4385 were first used for experiment 43, during ten weeks, so that the feeding of lead lasted a total of thirty-five weeks; similarly, the rats of 4291 were first used for ten weeks in experiment 42.

EFFECTS ON GROWTH

These varied considerably for the individual series. It will be more convenient to present these in groups; according to dosage, as in table 3. Typical curves are shown in figure 1.

It is seen that the doses of 0.00073 to 0.15 mgm. per kilogram per day interfere moderately but definitely with growth; the degree of interference increasing with the dosage, in this range, and with the duration of the administration. It is about equiva-

lent to the interference caused by arsenic, 0.00005 to 0.0005 mgm. per kilogram per day.

Curiously, however, this check was absent in the later series, with larger doses (0.3 to 1.22 mgm. per kilogram). The rats of each of these three series grew normally during the eight weeks

TABLE 2

Mean dosage of lead carbonate, and duration of experiments

CONCEN- TRATION OF LEAD- CARBONATE, PER KILO- GRAM OF FOOD	EXPERI- MENT NUMBER	NUMBER OF ANIMALS IN EXPERI- MENT	DURATION OF LEAD FEEDING	DOSAGE OF LEAD-CARBONATE PER KILOGRAM OF RAT PER DAY	TOTAL DOSE PER KILOGRAM MEAN WEIGHT OF RAT
mgm.			weeks	mgm.	mgm.
0.015	44	8	27	0.00073 (0.00052–0.00133)	0.137
0.05	43	7	27	0.0023 (0.0018–0.0035)	0.434
0.10	72	6	20	0.0059 (0.0031–0.0089)	0.826
0.15	42	7	27	0.0072 (0.0039–0.012)	1.36
1.5	4385	4	25	0.064 (0.046–0.072)	11.2
1.5	78	3	20	0.087 (0.055–0.160)	12.18
3.0	4291	3	17	0.15 (0.036–0.27)	17.85
5.0	121	3	8	0.30 (0.27–0.35)	16.8
10.0	122	3	8	0.66 (0.52–0.75)	36.96
20.0	123	3	8	1.22 (1.16–1.50)	68.32
	44N	1	2		

TABLE 3

Effects of lead carbonate on growth

MEAN DAILY DOSAGE PER KILOGRAM OF RATS	NUMBER OF SERIES OF EXPERI- MENTS	ACTUAL DURATION OF EXPERI- MENTS	WEIGHT CHANGES, DIFFERENCES FROM NORMAL WEIGHT, PER CENT PER WEEK AT END OF EXPERIMENT			WEIGHT CHANGES, DIFFERENCES FROM NORMAL WEIGHT, PER CENT PER WEEK AT END OF EIGHTH WEEK; MEDIAN
			Maximum	Minimum	Median	
mgm.		weeks				
0.00073–0.0072	4	27	+1.2	−0.72	−0.26	−0.17
0.064–0.15	3	20	−0.35	−1.6	−0.9	−0.3
0.3–1.22	3	8	+2.2	+1.2	+1.4	+1.4

of the experiment. It is very probably that they would have declined if the experiment had been continued longer; but the duration is not the whole explanation, for the other series declined within 8 weeks, as seen in the last column in table 3. One suggestive feature is that the rats used for the larger doses were
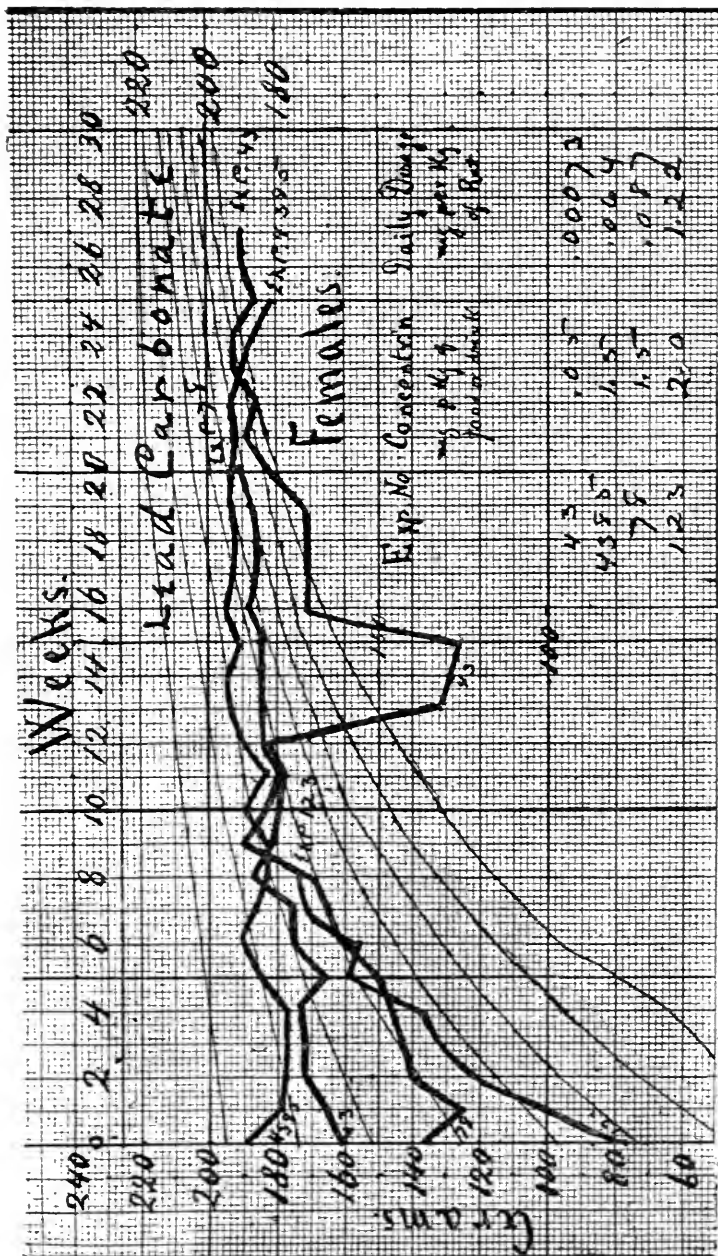
Fig. 1. Type Curves of Effects of Lead Carbonate on Growth

distinctly younger than the others. The observation illustrates
that dosage is not the only factor concerned in susceptibility
of lead-poisoning.

EFFECTS ON FOOD-CONSUMPTION

These are grouped, according to dosage, in table 4. The
number of experiments, and the duration are the same as in
table 3.

TABLE 4

Effect of lead carbonate on food-consumption

MEAN DAILY DOSAGE OF LEAD CARBONATE, PER KILOORAM OF RAT	MEAN DIFFERENCE IN FOOD CONSUMPTION FROM NORMAL STANDARD AT END OF EXPERIMENT	MEAN DIFFERENCE IN FOOD CONSUMPTION FROM NORMAL STANDARD AT END OF EIGHT WEEKS
mgm.	*per cent*	*per cent*
0.00073–0.0072	−2.8	+0.9
0.064–0.15	−2.0	+4.6
0.3–1.22	+24	+24

It is seen that the groups of experiments that showed deficient
growth also show deficient food consumption by the end of the
experiments; whilst the groups that did not lose in weight, had
very good appetites. The effect on growth is therefore quali-
tatively parallel with the effect on food-consumption. The
diminished appetite is not parallel to the dose, but is considerably
influenced by the duration of the experiment.

MORTALITY

This is shown in table 5. It appears rather high, 10 animals
dying in a total of 47 rats, fed on the leaded food. The table
shows that the fatality has no relation to the size of the dosage,
but depends apparently on the duration of the feeding. The
first deaths occurring after nine weeks. This is presumably the
reason why the higher doses were not fatal, since these were fed
only for eight weeks. If we take the animals fed longer than eight
weeks, the mortality is 10 out of 38, i.e., about one fourth. The
median time for the occurrence of death is ten weeks. Animals
that survived the seventeenth week did not die by the twenty-
seventh week.

The mortality of about 25 per cent may have been accidental; however, it is rather more probable that the lead feeding, extending beyond two months, weakens the resistance of the animals. This would be in harmony with the check of growth.

TABLE 5

Mortality

DOSAGE OF LEAD CARBONATE PER KILOGRAM OF RAT PER DAY	EXPERIMENT NUMBER	NUMBER OF ANIMALS	WEEKS OF FATALITIES	TOTAL DURATION	FATALITIES
mgm.				*weeks*	*per cent*
0.00073	44	8	11, 17	27	25
0.0023	43	7	10, 10	27	28
0.0059	72	6	9, 9, 12	20	50
0.0072	42	7	16	27	14
0.064	4385	4	0	25(+10)	0
0.087	78	3	9	20	33
0.15	4291	3	7	17(+10)	33
0.30	121	3	0	8	0
0.66	122	3	0	8	0
1.22	123	3	0	8	0

CONCLUSIONS

Rats to whose food lead carbonate was added in small doses (daily 0.0007 to 0.15 mgm. per kiloweight of rat) showed slight but definite check of growth and appetite. The effect starts within eight weeks, and increases with the duration of the feeding. No other definite symptoms occurred, even when the administration of lead extended over thirty-five weeks. The mortality was rather high, between nine and seventeen weeks; due probably to lowered resistance.

Another group of rats grew and ate normally although fed on larger doses of lead (0.3 to 1.22 mgm. per kilogram per day, for eight weeks). These animals were younger, which may possibly account for their resistance.

The daily dosage of lead in clinical human plumbism probably begins with $\frac{1}{5}$ to $\frac{1}{3}$ grain, 0.2 to 0.3 mgm. per kilogram. It is seen that much smaller doses, corresponding to as little as $\frac{1}{1500}$ grain per man, per day, are not harmless to rats. It is improb-

able that rats are much if any more susceptible to lead than is man. It is much more probable that these minute doses would also interfere with the nutrition and resistance of man, although they do not produce the clincial picture of plumbism. The results tend to throw some light on the wide hiatus that probably exists between clinical disease and harmlessness.

AN IMPROVED METHOD FOR USING PHENOLTETRA-
CHLORPHTHALEIN AS A LIVER FUNCTION TEST

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Phenoltetrachlorphthalein was studied pharmacologically in 1910 by Abel and Rowntree (1) who found that it was non-toxic when administered intravenously, and amongst its other characteristics, that it was excreted almost entirely by the liver. It was later employed as a test for hepatic function by Rowntree, Hurwitz and Bloomfield (2) and by Whipple, Mason and Peightal (3), who determined the amount present in the stools during the twenty-four hours following its injection. Subsequent clinical observations have proven rather unsatisfactory. Criticisms of this method are that it is time consuming and odious, that total stool collections are often inaccurate indices of the entire intestinal contents, and that the drug may be resorbed from the large intestine and be reëxcreted by the liver, this cycle continuing over a period of several days. Recently the estimation of its presence in the bile, utilizing the duodenal tube and the Meltzer-Lyons bile drainage, has been advocated. This method is disagreeable to perform and results must be interpreted in terms of negative values, i.e., dimunition in amount recovered, and delay in appearance time. Quantitative values are uncertain as the total bile excreted is not collected through the duodenal tube, varying amounts escaping into the small intestines.

Because of the fact that the liver is practically the only organ involved in its elimination, and because the dye appears in the urine when there is impaired output in the stools, it occurred to the writer that following the injection of phenoltetrachlorphthalein there might be retention in the blood, if liver function is impaired. Using the disodium salt, experimental studies

have been carried out on the rate of its disappearance from the blood stream in normal animals and in those with liver damage.

Whipple and Sperry (5) in a pathological study, showed that one or two hours of chloroform anesthesia produced varying degrees of central necrosis of the liver, and that within two weeks, repair as revealed in microscopic examination, was complete. Whipple, Mason and Peightal (3) determined the
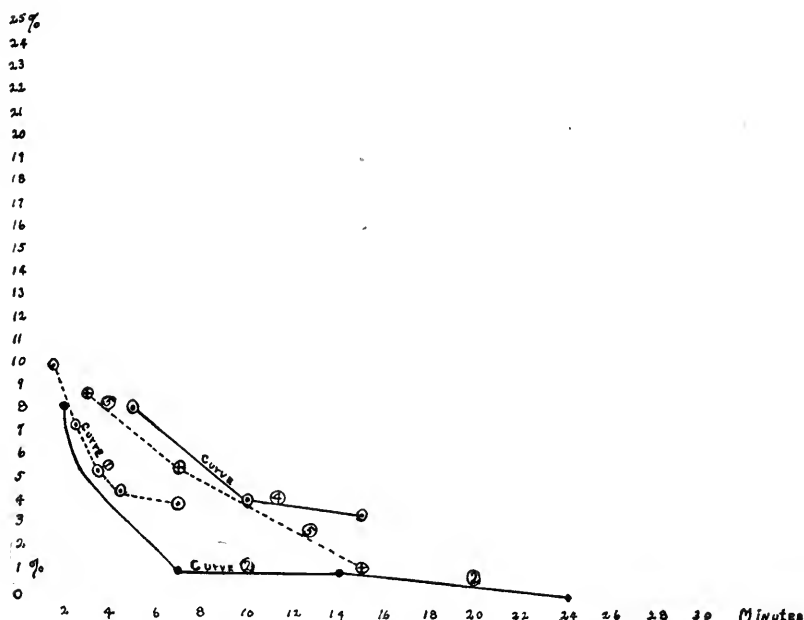
Fig. 1. Rate of Disappearance of Phenoltetrachlorphthalein from Blood of Normal Dogs

Curve 2, rabbit

dye excretion in the stools of dogs before and after chloroform poisoning, and found that the amount was greatly diminished, the impairment of excretion usually being proportionate to the extent of liver damage. In the non-fatal cases the dye excretion had returned to normal within a week's time.

A series of experiments is herein presented in which the dye was injected intravenously and its subsequent presence in the

blood estimated colorimetrically. Curves were obtained for normal dogs; the animals were then subjected to prolonged chloroform anesthesia and curves were again determined. The following technique was employed:

Under ether anesthesia the external jugular vein was exposed. Six cubic centimeters of blood was withdrawn as a standard for
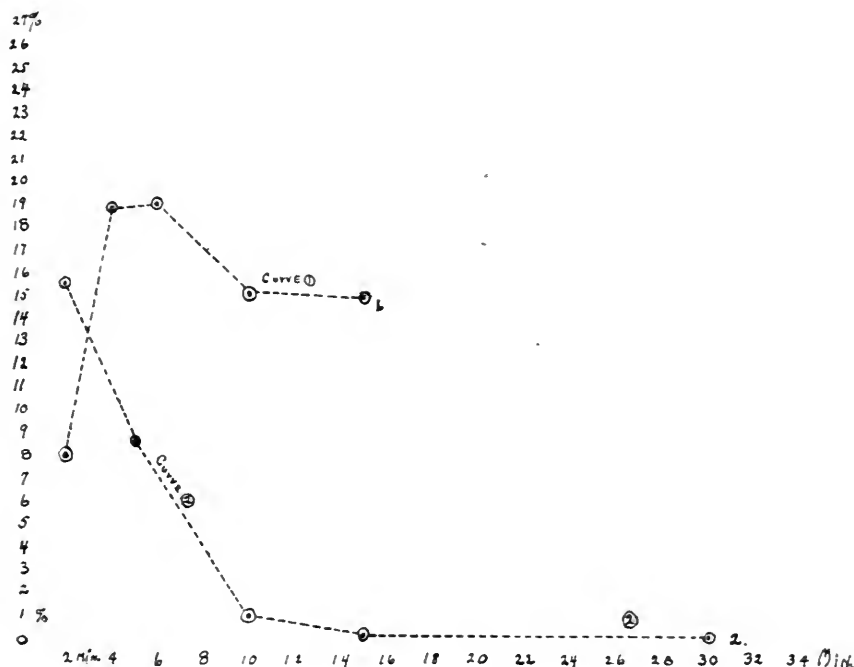
FIG. 2. DOG 3.   TWO HOURS CHLOROFORM ANESTHESIA

Dog ill for one day, then appeared normal.   Curve 1, twenty-four hours after. Curve 2, five days later.

colorimetric comparisons. Five miligrams per kilo of the dye (1 per cent aqueous solution) was then injected, following which 6 cc. of blood was withdrawn at frequent intervals. Blood was collected in oxalate and centrifuged immediately. The plasma was pipetted off and 3 to 5 drops of 10 per cent sodium hydroxide were added to each tube. A standard solution of the phthalein

was made up with 10 mgm. to 100 cc. of water. This was usually further diluted 1:5 or 1:10 when comparing with low percentages of dye in the plasma. Two cubic centimeters of standard plasma, 2 cc. of diluted dye, and 3 cc. of water were compared with 2 cc. of unknown plasma and 5 cc. of water.
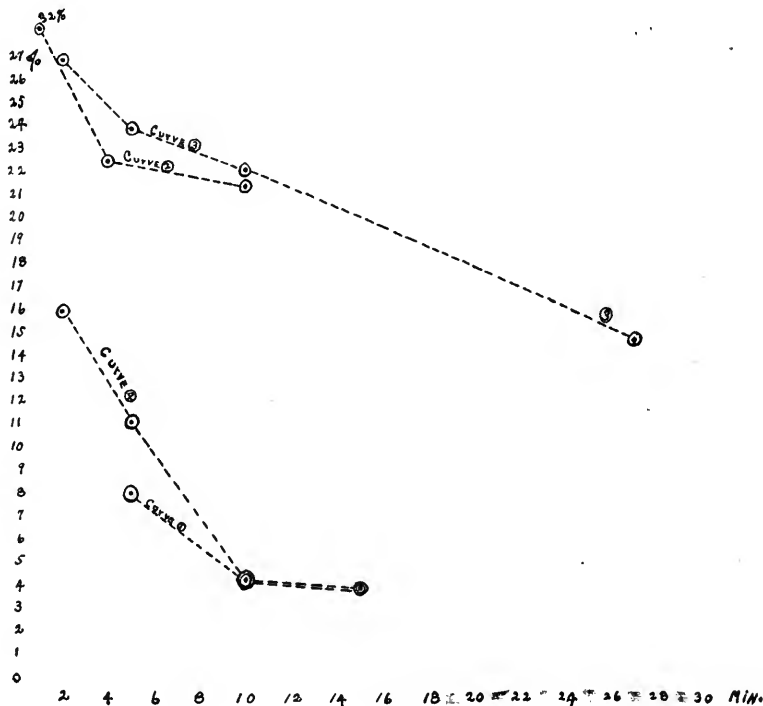
FIG. 3. DOG 4. TWO HOURS AND TWENTY MINUTES CHLOROFORM ANESTHESIA

Two days after, bile in urine and plasma. Curve 1, before anesthesia. Curve 2, twenty-four hours after. Curve 3, forty-eight hours after. Curve 4, seventy-two hours after.

The standard solution equals 100 per cent concentration, that is, if all of the dye remained in the blood approximately 100 per cent values would be obtained.

Curves obtained from normal dogs are shown in figure 1. It is seen that within from five to fifteen minutes only a trace of dye can be detected in the plasma. No dye appeared in the

urine of any of these animals. The question of conjugated or "colorless" dye has not been investigated. Dawson, Evans and Whipple (4) using a large series of dyes in studying blood volumes found in normal dogs, curves for phenoltetrachlorphthalein that were comparable with the above.

The above animals were subjected to chloroform anesthesia.
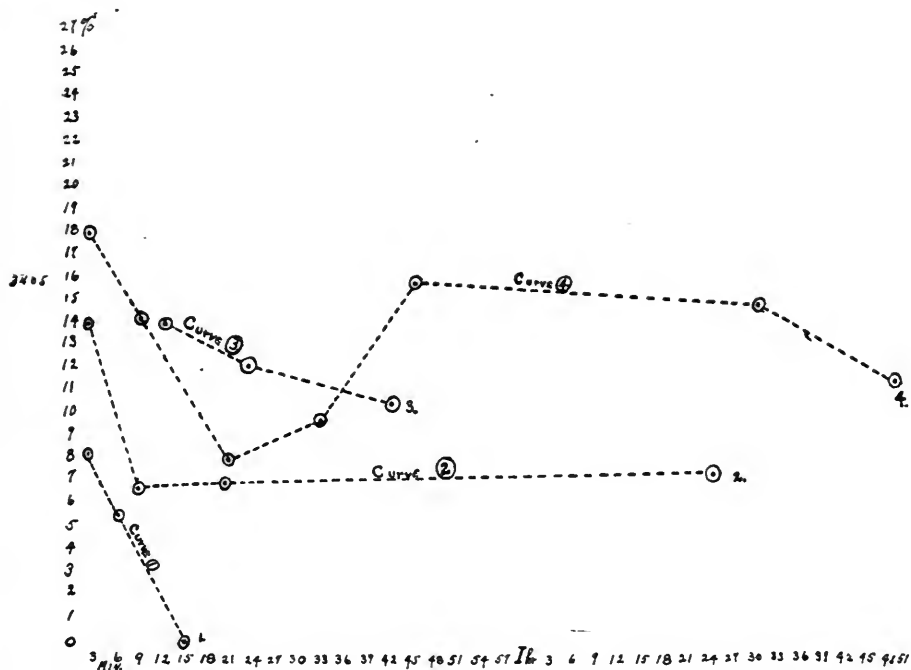
FIG. 4. DOG 5. TWO HOURS AND FIFTEEN MINUTES CHLOROFORM ANESTHESIA
    Curve 1, before anesthesia. Curve 2, eighteen hours after. Curve 3, forty hours after. Curve 4, five days after.

Dog 3 was anesthetized two hours; the reaction was not marked and after one day he did not appear ill. At no time did bile or dye appear in the urine, and the plasma was not bile stained. The blood curve for phthalein (fig. 2) showed moderate elevation twenty-four hours later, and a normal curve was obtained on the fifth day.

Dog 4 reacted more severely; on the following day (fig. 3, curve 2). The animal appeared quite ill, and the blood curve was markedly elevated. On the second day bile and dye were found in the urine, and the per cent of blood dye was even higher; on the third day no bile or dye were present in the urine and the dog looked less toxic (Compare curve 4).

Dog 5 before liver damage showed complete disappearance of the phthalein from his blood within fifteen minutes (fig. 4, curve 1). The following day chloroform was administered for two hours and fifteen minutes. On the third day the test was extended over a longer period of time, and remarkably high percentages of dye persisted as long as the experiment was continued (curve 2). Because of his excellent condition and because no bile or dye appeared in the urine, chloroform was again given for one hour on the afternoon of the third day. On the fourth day he appeared quite ill. Both phthalein and bile were present in the urine. The blood curve on that day presented a higher degree of dye retention (curve 3). On the sixth day of this experiment the bloods were withdrawn over a still longer period following the injection of the phthalein. The large amount of dye present in these specimens remained strikingly constant and showed little tendency to decrease; 11.2 per cent was found at the end of one hour and fifty minutes (curve 4). The urine contained a considerable amount of the dye, but only a trace of bile.

It has been necessary to discard all specimens showing hemolysis, as hemoglobin, if present in moderate amounts, interferes with colorimetric comparisons. From the above experiments it seems probable that this method offers promise of being developed into an accurate means of quantitatively estimating liver function. Further experimental and clinical studies are being undertaken to perfect its clinical application. By means of a series of standardized dilutions, the per cent of dye in the plasma could be determined similarly to the way in which phenolsulphonphthalein is estimated in the urine. If the dye can be separated from whole blood, small quantities of blood taken from the ear might be utilized.

CONCLUSIONS

Phenoltetrachlorphthalein has been injected intravenously and its subsequent concentration in the blood has been studied. In normal dogs there is an immediate rise to approximately 10 per cent, rapidly falling to only a trace or to complete disappearance within fifteen minutes. When the liver is damaged the amount of dye in the blood reaches 15 to 30 or more per cent, and remains elevated for a prolonged period, 11 per cent having been recovered almost two hours after injection. There is evidence that the curves obtained have paralled the degree of impairment of liver function, and it is believed that the method can be applied clinically as a quantitative test for liver function.

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COLLABORATORS

CONTENTS

INFORMATION FOR CONTRIBUTORS AND SUBSCRIBERS

BIOLOGICAL REACTIONS OF ARSPHENAMINE

III. ITS IMMEDIATE TOXICITY AS CONTRASTED WITH ITS LATE ILL EFFECTS, AND THE RÔLE OF AGGLUTINATION IN THE PRODUCTION OF THE FORMER

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In considering the toxic action of arsphenamine it is of interest to retrace the steps by which our present knowledge of the subject has been reached. The first investigations were done by the originator of the drug and his co-workers. Ehrlich and Hata (1) determined with infinite care the maximum dosis tolerata for different laboratory animals, and established the relation of this dose to the therapeutic efficacy of the drug. Its pharmacological properties were also carefully investigated by Hering (2).

Such data have in the case of most drugs proved sufficient to guide the clinician in the proper administration of the substance, but as experience with arsphenamine became greater it was evident that further factors than those which had been described in the experimental work might operate to produce ill effects. The clinician was therefore forced to develop and systemize his knowledge of these ill effects from his clinical material, and the results of these studies form a body of facts which, if carefully considered, fit in but poorly with the experimental studies of the toxicity of the drug. We cannot consider in detail the clinical reactions which have been observed. Suffice it to say that many of them fall into a group which may be called "early reactions" while others, from the delay in their devel-

393

opment, may be called "late."   In the first class must be spe-
cially mentioned those which occur almost immediately after or
even during the administration of the drug, varying from tran-
sient "nitritoid crises" to sudden collapse with death.   The
"delayed" effects include cases of dermatoses, nephritis, and
that affection of the liver which both anatomically and clini-
cally resembles acute yellow atrophy.

The result of these clinical observations has been a tremendous
stimulation to the pathologist, the pharmacologist and the
chemist, who have endeavored to determine the mechanism of
these untoward results.   It is impossible to even briefly cover
the entire field of these later studies, and we shall only consider
here those which have an immediate bearing on our personal
investigations.

From the functional disturbance noted in the early reactions
the impression is given of multiple embolism, and the source
of such an occurrence has been sought by one set of investiga-
tors.   Joseph (3) found that with acid arsphenamine precipi-
tation occurred in the blood stream with resulting embolism
in the lungs and other organs.   This was in accord with the
greater toxicity of acid arsphenamine as compared to the alka-
line form.   This idea was further developed independently by
Fleig (4), the results of whose studies are given in an extensive
monograph.

We believe one of the most important contributions of this
work, and one which has never received the attention due it,
is not only the division of the toxic effects in his experiments
into "early" and "late" ones, but of even greater importance,
the recognition that the mechanism of these two types of reac-
tion may be entirely different.   The early reactions Fleig believes
to be due to in vivo precipitation and embolism; the late effects
he considers due to the chemical properties of the drug.   He
therefore speaks of a "mechanical" and a "chemical" toxicity
and contrasts in some detail these two types.

More recently Danysz (5) has amplified the theory of toxicity
from in vivo precipitates, adding certain reactions which in
their relation to each other are analogous to the relation between

precipitinogen and precipitin. In this way he accounts for the occurrence of "anaphalactoid reactions" after previous harmless doses.

Another point in which we are especially interested is the anatomical evidence of thrombosis which has been found at autopsy, both in fatal human cases and in experimental animals. The earliest descriptions of fatal arsphenamine administration emphasized the occurrence of multiple thrombi, especially in the lungs. (Meissner) (6). Of the early experimental studies which were particularly concerned with this phase of the anatomical findings v. Marscholkó and Veszprémi (7) should be mentioned. Schindler (8) also emphasizes the importance of thrombosis. All of these investigators explain the thrombosis by the action of arsenic on the blood vessels.

While the present investigation was in progress Kolmer and Lucke (9) published their studies on the tissue changes following arsphenamine injections. Throughout all the experiments, in those in which both large and small doses were given, we find descriptions of peculiar thrombi of "conglutinated red cells" occuring in practically all the organs. The authors suggest that these thrombi are due to the hemolytic action of arsphenamine, which one of them (Kolmer) (10) had previously studied.

This brings us to the work of Karsner and Hanzlik (11) on in vivo agglutination of red cells as a cause of "anaphalactoid" reactions. Among other substances arsphenamine was studied and evidence produced which indicated that the agglutination might be the cause of certain of the disturbances noted, particularly lung inflation. Thrombi of agglutinated cells were also found in microscopic section of the tissues of such animals.

We have previously studied the agglutinating action of arsphenamine in vitro (12) and now propose to determine if a similar process may occur in vivo. Two facts make such experimental determination necessary. First the well known discrepancy often observed between in vitro and in vivo phenomena and secondly the possibility that the blood plasma may inhibit the agglutinating action of the arsphenamine by its action as a protective colloid.

The plan of the experimental work has been as follows: A first set of experiments was done to determine the cause of immediate death when large doses are given, and also to establish at least approximately the size of such a dose. With these facts determined, another series of animals was given smaller doses, some large enough to produce a definite reaction; others, not. The animals were then killed after varying periods, and a search made by several methods for those occurrences which had been found to an exaggerated degree in the previous experiments with large doses. A final series was given comparatively small doses repeatedly in order to produce toxic symptoms or death after a week or several days.

In all experiments the disodium salt of arsphenamine was used, prepared in a 2 per cent aqueous solution according to the standard method of the United States Hygenic Laboratory. The animals used throughout were rabbits. The arsphenamine was injected intravenously with a syringe at an approximate rate of 1 cc. per minute. The total number of experiments performed was fifty-nine. Of these, twenty animals were used in the experiments detailed in parts I and III, seven in those of part II, and thirty-two in part IV.

I. THE EFFECT OF SINGLE LARGE LETHAL DOSES

If a rabbit be given a 2 per cent solution of disodium arsphenamine intravenously, a point is reached when the animal becomes dyspnoeic, gives a loud cry, sinks to the floor and convulsions of the entire body set in, often of an opisthotonic type. In a few seconds the respiration becomes even more labored and finally ceases abruptly. The heart may continue to beat for some little time. Occasionally the convulsive phenomena are less striking and the animal lies prone, its legs so weak as not to support the body, its head hanging limply to one side. The general picture of such a seizure is identical with that due to multiple fat embolism after the intravenous injection of oil.

The dose necessary for the production of such effects depends somewhat on the speed of the injection. As our purpose was to produce an immediate death, no particular care was taken

to give the arsphenamine very slowly. A constant rate of injection was used, however, throughout the experiments of all series, 1 cc. per minute, so that the results in all experiments are comparable. Table 1 gives an abstract of the effect of doses of different size.

It will be seen from the table that doses larger than 0.23 grams per kilo produce immediate death, i.e., within ten minutes of the end of the injection. Below this figure the results vary somewhat, some animals showing a definite reaction and dying in one to two hours. Others show little evidence of damage while living, yet when killed they present definite lesions at autopsy. These latter experiments are described in detail in the third section of this paper.

Anatomical findings

A rabbit which had died immediately following a large injection of arsphenamine was opened at once. The vena cava inferior below the liver was cut and blood collected in test tubes, both with and without isotonic sodium citrate. If the blood from the test tube containing citrate is poured on a glass plate, it is easily seen that it shows a marked agglutination (fig. 1). The agglutinated clumps are even more strikingly demonstrated if a drop of the citrated blood is placed beneath a cover slip and examined with the microscope (fig. 2). The clumps are composed of red cells alone, which are heaped on each other indiscriminately, forming closely united masses in which the individual cells, though still visible, are markedly distorted. Each clump has the appearance of a cluster of wax cells which has been slightly warmed with a resulting sticking together and deformation of their shape. There is nothing in their appearance that resembles rouleaux formation, nor is there any evidence of coagulation. Similar small bits of really clotted blood are definitely bound together by a mass of fibrin which has a finely granular appearance. Further evidence that coagulation does not play a part in the production of these clumps is found in the fact that the blood from the vena cava drawn without citrate does not clot readily. Samples from such animals have been kept for days without the occurrence of coagulation.

TABLE 1

Effect of single injections

EXPERIMENT	RABBIT NUMBER	DOSE PER KILO	REACTION	TIME OF DEATH	BLOOD FROM VENA CAVA			AGGLUTINATION IN EAR VEIN	CLUMPS IN PERFUSION FLUIDS		GROSS ANATOMICAL FINDINGS
					Agglutination	Coagulation*	Hemolysis†		Spleen	Lungs	
1	51	0.42	Marked	End of injection	+++	0	±				Lungs, congested; many petechiae; spleen, normal size
2	19	0.40	Marked	End of injection	+++	0	±				As above, see figure 5
3	20	0.32	Marked	End of injection	+++	0	±		+++	+++	As above
4	21	0.30	Marked	End of injection	+++	0	±		+++	+++	As above
5	23	0.30	Marked	End of injection	+++	0	±		+++	+++	As above
6	48a	0.28	Marked	End of injection	+++	0	±				As above
7	50	0.27	Marked	End of injection	+++	0	±				As above
8	47	0.27	Moderate	Killed 3 hours	0	−	±	+ 15 minutes − 30 minutes	+++		Lungs, few petechiae, moderate edema; spleen, enlarged, soft and congested
9	25	0.23	Marked	End of injection	+++	−	±				Lungs, many petechiae; spleen, normal size
10	22	0.23	Moderate	Killed 45 minutes	+++	−	±		+++	+++	Lungs, as above; spleen congested

11	24	Moderate	Killed 3 hours	0	±	±	− 20 minutes	+++	+++	Lungs, few petechiae; spleen, enlarged, soft and congested
12	26	Moderate	2½ hours	0	−	±	+ 20 minutes / − 1 hour	+++	+++	Lungs, few petechiae, marked edema; spleen, swollen, congested and soft
13	28	Slight	Killed 6 hours	0	−	0	+ 10 minutes / − 1 hour	+++	+++	As above
14	29	Slight	Killed 5 hours	0	±	±		+++	+	As above
15	27	Marked	End of injection	+++	−	0	− 10 minutes	+++	+	Lungs, many petechiae; spleen, negative
16	49	Marked	Killed 4½ hours	0	±	0		+++	+++	Lungs, many petechiae; spleen, swollen, soft and congested
17	48	Moderate	Killed 2½ hours	0	±	±		+++	+	As above
18	46	Slight	Killed 2½ hours	0	±	±	− 10 minutes	+++		As above
19	33	Slight	Killed 3½ hours	0	±	±	− 10 minutes	+++		As above
20	32	Slight	Killed 3 hours	0	±	±	− 10 minutes	++	+	As above

(values 0.23, 0.23, 0.22, 0.22, 0.21, 0.19, 0.18, 0.16, 0.16, 0.11 appear in the second column)

* 0 = no coagulation in two hours.
− = definite delay in coagulation.
± = gross method of observation showed indefinite results.
† ± = plasma or serum slightly tinged with hemoglobin, probably the result of mechanical damage to cells during handling.

FIG. 1. RABBIT 48A, 0.28 GRAM OF ARSPHENAMINE PER KILO

Died of typical acute seizure at end of injection. Blood from inferior vena cava in isotonic sodium citrate, showing extensive agglutination. The photograph is natural size.
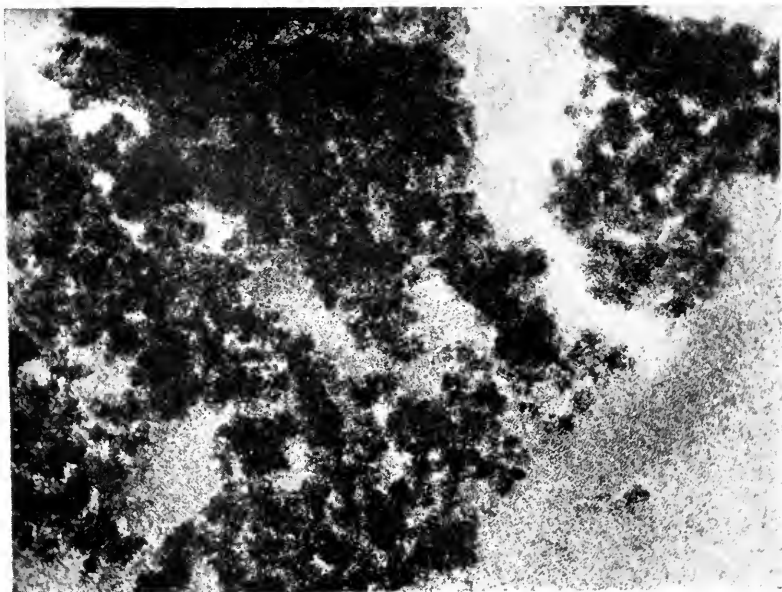
FIG. 2. RABBIT 20, 0.32 GRAM ARSPHENAMINE PER KILO

Sudden death at end of injection. Microscopic preparation of citrated blood from inferior vena cava. Bausch and Lomb ocular 1, objective $\frac{2}{3}$.

Similar clumps of agglutinated cells are found on both sides of the vascular system, both in the right side of the heart and in the left ventricle.

In the presence of such extensive agglutination, embolism of small vessels and capillaries is inevitable. The anatomical evidence of such embolism is found in practically all organs, though in certain of them the process is more easily demonstrated than in others. This is particularly true of pale tissues, such

Fig. 3. Rabbit 48a. Sudden Death Following 0.28 Gram Arsphenamine per Kilo

Inferior surface of the frontal lobes of brain, the optic chiasma at the upper limit of the picture. Practically all the vessels are filled with emboli of agglutinated cells. Some of the smaller ones are invisible except for the dark spots formed by the emboli. Zeiss compound ocular 6, objective planar, series 1a, no. 4.

as the brain or thymus gland. Here with the naked eye, or better by the aid of a hand lens, one can make out clumps and dotted points in the small vessels which run on the surface (fig. 3). An even more striking demonstration may be made by stretching a loop of intestine with its attached mesentery on the stage

of the microscope and examining it with low power.   Clumps
of agglutinated cells may be seen in the arterioles and veins,
often lodged in the former at a point of bifurcation (fig. 4).

The lungs, as would be expected, show in all cases most strik-
ing evidence of the multiple emboli.   The entire pulmonary
tissue is tremendously congested, and on this red background
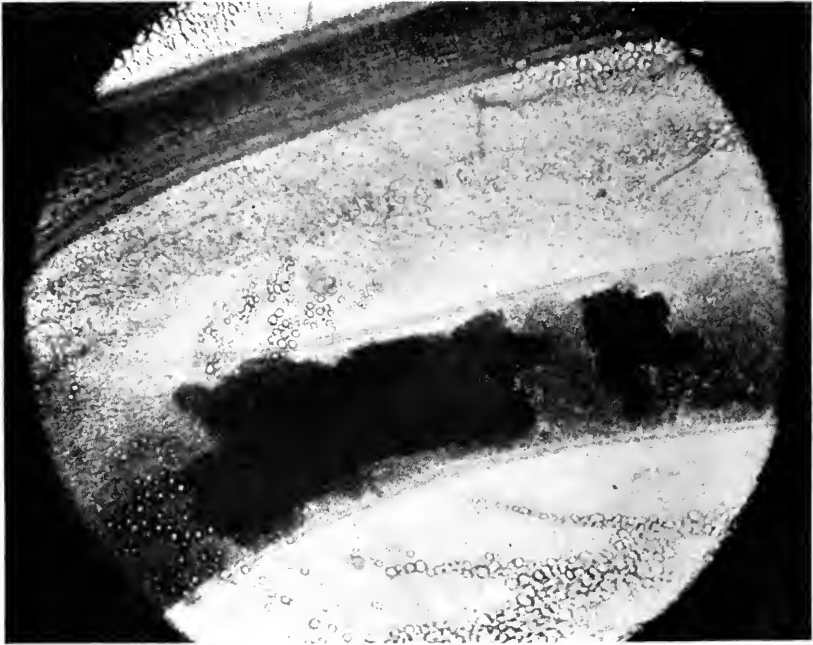
FIG. 4. RABBIT 51. SUDDEN DEATH FOLLOWING 0.42 GRAM ARSPHENAMINE
PER KILO

Vessels in mesentery of small intestine.   In the large vein are two large clumps
of agglutinated red cells.   Bausch and Lomb ocular 2, objective $\frac{2}{3}$.

are an infinite number of just visible dark red spots.   Grossly
they have the appearance of petechial hemorrhages (fig. 5).
The right side of the heart is dilated, sometimes to an extreme
degree.   The remaining organs, spleen, kidney, liver, and heart
muscle show little gross evidence of embolism on account of the
lack of contrast in these darker tissues.

FIG. 5. RABBIT 19, 0.40 GRAM ARSPHENAMINE PER KILO

Death with typical acute seizure at the end of injection. There is a diffuse congestion of both lungs and this dark background is closely studded with small embolic petechiae.
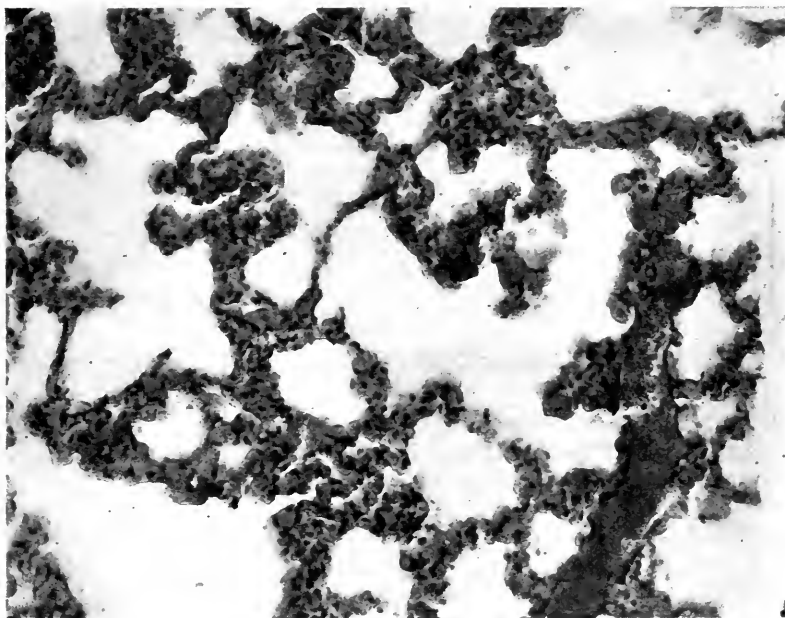
FIG. 6. RABBIT 20. DIED ACUTELY FOLLOWING 0.32 GRAM OF ARSPHENAMINE

The capillaries of most of the septa are filled with masses of agglutinated red cells. In contrast, note the alveolar walls which do not contain clumps and which are therefore of normal thickness. Bausch and Lomb ocular 1, objective $\frac{2}{3}$.

403

For microscopic study tissue from the more important organs was fixed in 10 per cent formalin and in Orth's fluid, and paraffin sections made. Hematoxylin and eosin and Giemsa's stains were used. It is often a matter of considerable difficulty to distinguish between the agglutinated masses of red cells which form during life and those red cells which are fused together by the action of the fixing solution. In our experiments the finding
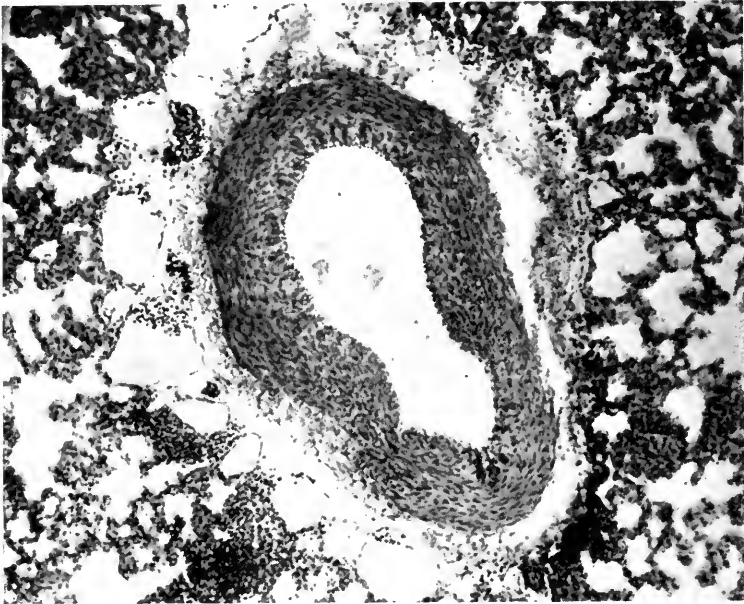
FIG. 7. RABBIT 27. IMMEDIATE DEATH FOLLOWING 0.21 GRAM ARSPHENAMINE PER KILO

The pulmonary capillaries are crowded with agglutinated red cells. Around the artery are seen the dilated lymphatics filled with fluid and a few leucocytes. Bausch and Lomb ocular 1, objective $\frac{2}{3}$.

of clumps before fixation served as a control for this possible error.

The sections of the lungs were most instructive, for here we have an abundance of capillaries poorly supported by the thin alveolar walls. An obstruction of such a vessel produces a marked and often fairly well circumscribed bulging which pro-

jects into the open space on each side. Such an appearance is noted in our specimens (fig. 6). The larger vessels are dilated and filled with red cells so closely packed that it is impossible to decide whether they are clumped together or not. The capillaries of the septa are in places also packed with cells and in other regions collapsed. In some capillaries the cells are definitely arranged in agglutinated masses forming typical "thrombi of conglutinated red cells." Although there is only a slight edema of the alveolar spaces, the perivascular lymphatics are often distended with clear fluid, in which are a few white cells (fig. 7).

Sections of other organs show the agglutinated cells less clearly, because the capillaries in them are not so easily distended. In the liver, spleen, kidney, brain and heart muscle one can, however, make out aggregations of clumped cells. In none of the organs were noted any tissue changes, such as parenchymatous degeneration.

II. THE EFFECT OF INTRAVENOUS INJECTION OF RED CELLS PREVIOUSLY AGGLUTINATED BY ARSPHENAMINE

To determine the relative importance of embolism by agglutinated cells and of a toxic action from the arsphenamine, the following experiment was performed. It is the experiment devised by Flexner (13) to meet a similar problem in his studies on ricin poisoning. A rabbit's cells were washed twice with 0.9 NaCl, and made up to a 10 per cent suspension. To 37 cc. of the suspension was added 1.9 cc. of 2 per cent arsphenamine solution. Marked agglutination resulted. Of this mixture 25 cc. were injected into the rabbit's ear vein. The result was a seizure of exactly the same type as described previously following the injection of a large dose of arsphenamine. At autopsy the same embolic lesions were found in the lung, but none on the arterial side of the circulation. The blood in the inferior vena cava was also free of clumps and clotted promptly. Microscopic examination of the pulmonary tissue showed essentially the same picture as described previously, including the perivascular edema (fig. 5).

Death in this experiment, which was repeated several times, was evidently due to multiple pulmonary emboli. The amount of arsphenamine injected, 0.025 gram per kilo, is insufficient to cause any immediate reaction.

III. REACTIONS FOLLOWING LARGE BUT NOT IMMEDIATELY FATAL DOSES OF ARSPHENAMINE

That arsphenamine in large doses causes extensive in vivo agglutination, and that this process in the cause of the acute immediate death which follows the administration of such amounts, has been established. It now remains to determine if a similar process occurs after doses just large enough to produce the external evidence of a reaction, and after even smaller ones which are followed by no obvious change in the behavior of the animal. In the present series rabbits were given decreasing amounts, their behavior noted, and their blood examined for agglutinated red cells.

Although it is a comparatively simple matter to find the clumps of agglutinated cells by direct observation when they are present in enormous numbers in the blood, as is the case following a large immediately fatal dose, it is obvious that more refined methods will be required when the clumps are so few in number as to produce little damage to the organism. Another difficulty arises in the fact that if the animal lives for some time the clumps are removed from the circulating blood by such organs as the lungs and the spleen. One can examine sections of these organs for the presence of clumps of red cells, but the disadvantage of this method in which the cells are studied after they have been acted upon by fixing fluids has already been mentioned.

After those doses to which there was a definite reaction on the part of the animal, a drop of blood from the ear vein was mixed at once with an equal amount of citrate and examined microscopically. In those animals which had received even smaller doses, a large amount of blood was collected in citrate from the vena cava inferior and diluted several times with the same fluid. This suspension was then centrifugalized at very low speed for a short time, the supernatant fluid removed, and the sediment examined microscopically

As has been stated, if the animal lives some time the clumps are removed from the circulation by such organs as the lungs and spleen, and advantage may be taken of this by examination of these depots where clumps accumulate. A method similar to that devised by Rous and Robertson (14) for the collection of blood cells from various organs was used. This consists of perfusion of the organ with isotonic sodium citrate, either by way of its vascular channels, as in the case of the lungs, or by
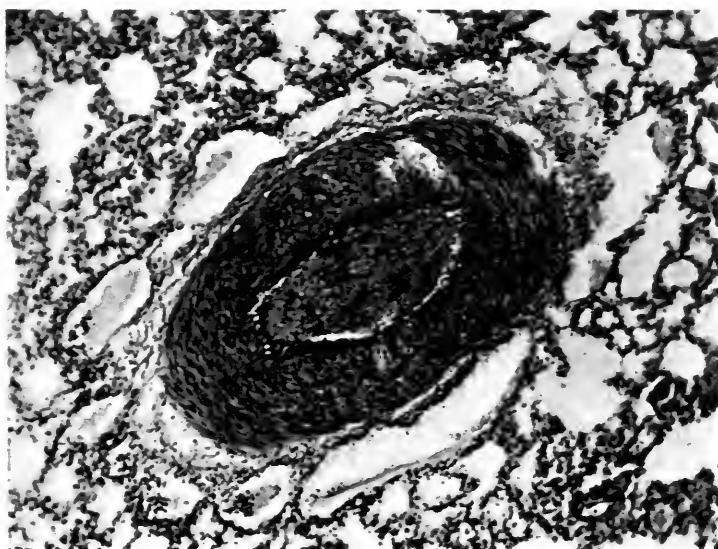
FIG. 8. RABBIT 54. DIED SUDDENLY AFTER AN INJECTION OF 25 CC. OF RED CELLS PREVIOUSLY AGGLUTINATED WITH ARSPHENAMINE

The lumen of the artery is filled with a thrombus of agglutinated red cells and leucocytes. The same dilatation of the perivascular lymphatics is seen as was observed in the previous specimen. Bausch and Lomb ocular 1, objective $\frac{2}{3}$.

thrusting a needle into the pulp of the organ, when the other method is impractical, as in the case of the spleen. The perfusion fluid is then centrifugalized at low speed, and the sediment examined with the microscope.

The blood serum of all animals was also examined for free hemoglobin, as an evidence of in vivo hemolysis.

Table 1 gives a résumé of the findings in our experiments. A detailed description of typical experiments is given below.

Marked reaction. *Rabbit 26.* Given 0.23 grams per kilo of 2 per cent di-sodium arsphenamine at 10:20 a.m. One minute after the injection a definite palsy of the head developed, the animal's front legs became weak so that its body slipped to the floor and slight dyspnea developed. There was a gradual recovery from this state during the next half hour. From that time on the animal presented no symptoms but remained very quiet. Ten minutes after the injection a drop of blood from a vein in the ear which had not been injected showed many small clumps of agglutinated cells on microscopical examination. The vessels of the ear were at this time markedly contracted, but gradually resumed their normal calibre during the next twenty minutes. Microscopical examination of the ear vein blood was repeated after twenty minutes and a few clumps of red cells found, while in another examination after one hour none were present. At 1:00 p.m., two hours and a half after injection, the rabbit died. The abdomen was opened at once. The blood from the inferior vena cava showed no gross evidence of agglutination when drawn in citrate, now could any clumps be found on differential centrifugalization. The spleen was definitely enlarged, dark red in color and soft. The lungs showed a marked hyperemia and edema with many small dark round spots scattered indiscriminately throughout the pulmonary tissue. Both the spleen and lungs were perfused with isotonic citrate immediately on removal, and the washings centrifugalized at low speed. The sediment of both showed a large number of clumps of red cells, the spleen the greater number, probably on account of the greater ease with which its blood sinuses can be washed out. The emboli of red cells from the spleen and lung presented a somewhat different appearance from those seen when death had occurred immediately. Some were of the type described previously, being composed of a solid mass of somewhat distorted fused red cells. Many, however, were more or less covered with a layer of granular white cells which adhered firmly to the central red clump. Pressure on the cover glass dislodged some of these cells, while others were firmly united to the embolus. This type of embolus was even better developed in some animals which had lived longer, and will be mentioned again in later experiments.

Microscopic sections of the heart, brain, liver, kidney, lung and spleen showed emboli of agglutinated cells in greater or less number, being greatest in the vessels of the lungs and the sinuses of the spleen. In

the former the capillaries of the septa are filled with masses of fused red cells as described above, but in these sections there was also a considerable admixture of polymorphonuclear leucocytes. The alveolar spaces contain a large amount of clear fluid in which there is also a beginning extravasation of polymorphonuclear leucocytes. Similar clear fluid is seen in the bronchioles and the small bronchi and in the perivascular lymphatics (fig. 9.) In the spleen the sinuses are so
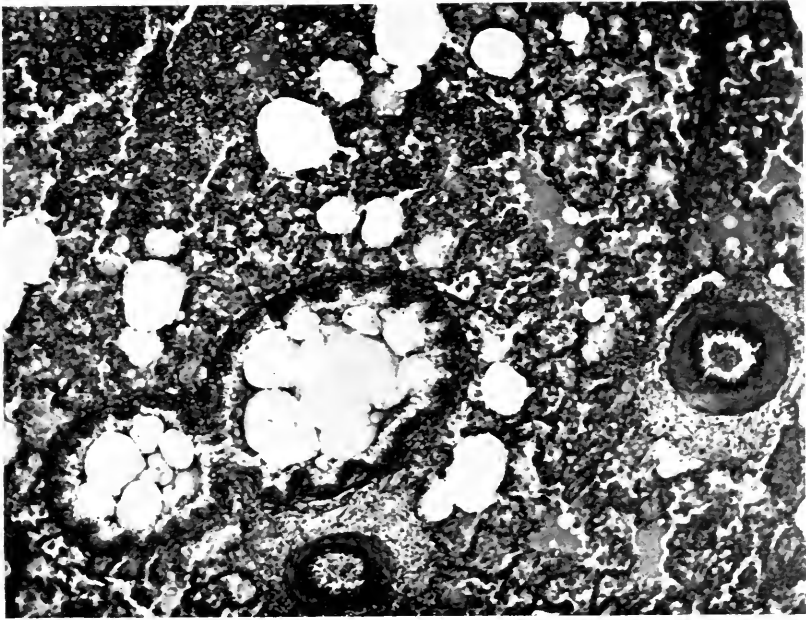
Fig. 9. Rabbit 26.   Died Two and One-half Hours After the Injection of 0.23 Gram Arsphenamine

Practically all the alveolar spaces are filled with fluid and there is a considerable perivascular edema around the two small arteries. A small bronchus is also filled with fluid and in the upper left corner there is an extravasation of leucocytes into the alveolar spaces. Bausch and Lomb ocular 1, objective ⅔.

greatly distended with masses of fused red cells that it is difficult to separate the individual aggregates. Scattered among these masses are also seen a considerable number of polymorphonuclear leucocytes.

The other organs show occasional clumps of red cells but no other lesions.

Slight reaction. Rabbit 46. Given 0.16 gram arsphenamine **per** kilo at 2:12 p.m. Slight reaction consisting of moderate dyspnea developing three minutes after injection. The animal, which had been very lively previous to the injection, remained very quiet afterwards, but showed no other signs of ill effect. No agglutinated clumps could be found in the blood from the vein of the uninjected ear at five, fifteen, and thirty minutes following the injection. At 4:30, two hours and thirty minutes after the injection, the animal was killed with chloroform. Blood from the vena cava inferior showed no agglutinated clumps on centrifugalization in citrate solution. It also clotted fairly promptly. The spleen was definitely enlarged, about one and a half times the normal size, dark red and quite soft. In the sediment of the citrate perfusion fluid from it were found many clumps of agglutinated cells, covered more or less completely with leucocytes. The red cells in these clumps were even more intimately fused than previously so that their outlines could be made out only with difficulty, except as they appeared as rounded projections on the free surface of the clump. The comparison previously made with a partly melted mass of wax cells is even more applicable here, where in the center the outlines of the individual units is entirely lost. Such a clump often forms the central nucleus, a golden brown from its hemoglobin, of what may now be called a thrombus, the pale periphery of which is formed of leucocytes. Giemsa stains of these thrombi showed that there was also a considerable number of blood platelets in the outer layer of leucocytes. No evidence of phagocytosis of red cells or of clumps was seen.

The lungs were hyperemic throughout and showed a moderate number of small embolic red points. It was found that perfusion of the lungs was often unsatisfactory if the animal had lived some time after the occurrence of the embolic process, as it was very difficult to dislodge the clumps from the capillaries of the septa. Such was the case in this animal in which the attempt was unsuccessful. The gross evidence of emboli and the microscopic demonstration of the embolic clumps in the capillaries was, however, quite definite.

The other organs were all negative to gross examination. Microscopic section of tissues showed much the same picture as has been described for the previous rabbit, the number of emboli and the resulting circulatory disturbances in the lung being less marked. In other organs than the spleen and lung it was difficult to definitely identify agglutinated clumps. No tissue lesions could be made out.

IV. THE EFFECT OF REPEATED DOSES OF ARSPHENAMINE

The final series of experiments was performed for the purpose of contrasting certain tissue lesions which require longer periods for their development and to determine if possible if these could be due to a type of action other than that already described. These lesions have been described by other investigators and as our results are only confirmatory of these findings they will be given very briefly.

Repeated injection of large doses—0.1 gram per kilo. These animals, twenty in number, were given two to three doses of 0.1 gram of arsphenamine per kilo. Sixteen received the drug in 2 per cent solution, and the other four in 0.5 per cent. Of the former all but one died, six very suddenly during the course of the third injection. Of the latter two died, one suddenly during the second injection. These sudden deaths which resembled the type which follows the injection of a large immediately fatal dose, such as has been described previously, were due to massive intravascular agglutination. The details of this phase of the experiments, together with the reason of the agglutination from these comparatively small doses, will be considered at another time.

The lesions found in those animals which received the 2 per cent arsphenamine were practically identical with those described by Kolmer and Lucke (15) except that in those animals which died suddenly during the injection the evidence of agglutination and embolism was more marked.

The lungs showed many clumps of agglutinated cells throughout the pulmonary capillaries, as well as a marked passive congestion of the larger vessels. In most cases there was also a marked edema of the perivascular lymphatics and of the alveolar spaces. The bronchi were also filled with fluid. In some specimens there was a definite leucocytosis of a polymorphonuclear type in the capillaries and also a beginning exudation of these leucocytes into the tissues and alveolar spaces.

Sections of the spleen showed a marked dilatation of the venous sinuses, which were crowded with red blood cells and in places with clumps of agglutinated cells. Phagocytosis of red cells and pigment deposits was not marked.

The liver showed only slight evidence of parenchymatous degeneration. The protoplasm of the liver cells in certain areas was dis-

tinctly granular, and occasionally pyknotic nuclei were seen. Marked fatty degeneration of the liver cells was not present.

Sections of the heart showed little evidence of damage. Occasional clumps of agglutinated red cells were seen in the capillaries, but no definite lesions in the muscle cells.

Sections of the adrenals were negative except for emboli of agglutinated cells. Fat stains for the lipoid content of their cortices were so variable that no definite statement can be made as to the relative amount of fatty material in different specimens.

Sections of the kidney showed the most marked lesions. These were identical with those described by Kolmer and Lucke (15), consisting of vascular lesions, such as embolism from agglutinated red cells and congestion, and also of extensive degeneration and necrosis of the epithelium of the convoluted tubules. Calcification of the necrotic material was also seen in some specimens. The distribution of the necrosis was quite diffuse in our specimens and seemed to bear no relation to the vascular supply.

In animals which received an equal amount of arsphenamine but in a dilute form, the agglutinative phenomena were much less marked, yet the degeneration in the kidney and liver were equal to that found in the previous experiments.

The lesions occurring in this series of experiments, other than the presence of emboli of agglutinated red cells, consist therefore in vascular congestion and epithelial degeneration with necrosis and calcification in the kidney and slighter parenchymatous degenerations in the liver.

Repeated injection of small doses—0.04, 0.03, 0.01 gram per kilo. Smaller doses were repeatedly injected in the hope that tissue lesions would be produced without any evidence of agglutination of red-cells and embolism. The results of these experiments may be briefly summarized as consisting of slight parenchymatous degeneration in the kidney and liver. In neither organ was definite necrosis of the epithelium seen. We were unable to definitely determine if any clumps of red cells were present. Occasional masses of coalesced corpuscles were found, but a similar appearance, as we have mentioned before may result from the action of fixing fluids.

SUMMARY AND DISCUSSION

The injection of a large fatal dose of arsphenamine results in disturbances which in their general characteristics are typical of multiple embolism. The reaction of the animal as well as

the form of death is almost identical with that which follows the injection of oil or liquid fat into the ear vein. Anatomical investigation of such animals shows that the cause of these disturbances is in fact multiple embolism in both the pulmonary and peripheral circulation and that the emboli in this instance are clumps of agglutinated red cells.

The result of this widespread embolism varies with the number of occluded capillaries. Following large doses, death results so quickly that little time is given for the development of secondary lesions. In the lungs, the almost complete stoppage of the pulmonary capillaries produces a tremendous congestion of the larger vessels, which extends back and causes in turn a dilatation of the right ventricle. The perivascular lymphatics are distended with clear fluid, but no extensive collection of fluid is found in the alveolar spaces. The organs connected with the peripheral circulation show the same congestion to a lesser degree, as here the number of emboli is smaller and the vascular obstruction correspondingly less.

On the other hand, if the animal lives for some hours, these changes due to circulatory disturbances become more pronounced, especially in those organs where the emboli are filtered out of the circulating blood. From the peripheral veins and the pulmonary arteries the emboli are carried into the lungs. The perivascular edema is increased and there is also a considerable collection of fluid in the alveolar spaces and in the bronchi. A beginning exudation of leucocytes is also often noted. From the pulmonary veins and the peripheral arteries clumps are lodged in the capillaries of all organs. It is in the spleen that they collect in largest number and where they produce the greatest morphological change. At a later period this organ regularly shows an enlargement with congestion and softening, which on examination proves to be due to a tremendous engorgement of its venous sinuses with clumps of red cells.

Changes also occur in the structure of the agglutinated red cells. In the earlier stages these clumps are composed of red cells alone, but after a few hours a considerable number of leucocytes are seen closely adherent to their surface. After some

time the bulk of the embolus may be composed of leucocytes and blood platelets in the center of which may still be seen the original nucleus of agglutinated red cells, so that final structure is much like that of a typical coagulative thrombus. Similar changes in agglutinative thrombi have been described by Flexner (16) and Aschoff (17). From our studies of the process of agglutination in vitro (18) we know that the arsphenamine is strongly bound by the red cells, and it may be that the collection of leucocytes around these clumps of red cells is augmented by a chemotactic property of the drug.

It is interesting to note in passing that although the end result of these processes is a product much like a coagulative thrombus, the coagulation of the blood is much decreased, or even prevented, by the administration of a large dose of arsphenamine. The blood of such animals, though markedly agglutinated, often remains permanently fluid, while in other cases the coagulation time is greatly prolonged. There can be, therefore, no possibility that coagulation plays any part in the formations of the original clump. A similar paradox, apparent "thrombi" in organs with decreased coagulability of the blood, has been noted in death following snake venom, and Wells (19) has suggested that in such cases the "thrombi" are in fact due to agglutination rather than to coagulation. Such is certainly the case in our experiments. A further study of this phase of the action of arsphenamine is now being made by us.

After moderate doses of arsphenamine evidence of the same intravascular agglutination was found by the perfusion of organs, such as the lungs and spleen, where the clumps collect. As these animals lived some time after the injection, the secondary changes, such as edema of the lungs and enlargement of the spleen, were most marked in them.

Although the presence of these embolic processes would seem to account for that type of reaction which is observed in the animal, the possibility still remains that some part may be played by a direct toxic action of the drug. An attempt was therefore made by means of an experiment devised by Flexner (26) for a somewhat similar problem, to determine

the relative importance of the purely physical effect of embolism on the one hand, and a toxic action of the drug on the other. This consists in the intravenous injection of an animal's red cells which have been previously agglutinated by arsphenamine. In this mixture of agglutinated cells the amount of arsphenamine is so small as to be negligible, so that any reaction noted must be considered the result of embolism of the clumped cells. By such means the essential reactions and anatomical findings that follow the injection of a large dose of arsphenamine were reproduced. As the lungs in such experimets present a barrier to the entrance of the clumps into the peripheral circulation, the changes noted in the spleen after an injection of arsphenamine did not occur.

The action of arsphenamine has been recently studied by Jackson and Smith (21) and later by Smith (22) alone. In both studies the most striking change observed was a marked rise in the pulmonary blood pressure, a dilatation of the right side of the heart and a drop in the arterial pressure. These phenomona they believe due to vascular obstruction in the pulmonary circulation, due in part to a direct action of the drug on the pulmonary vessels and also to the formation of emboli of precipitated arsphenamine in the pulmonary capillaries. The occurrence of such a precipitate in the blood serum may be directly demonstrated with acid arsphenamine, but not with the disodium salt. Our experiments, however, show that with this latter preparation the occlusion is due to embolism from agglutinated red cells, for such agglutination occurs after the adminstration of amounts far below the immediately fatal dose. Again we see the analogy between the result of arsphenamine injections and fat embolism. One has only to compare Bissel's tracings (23) of the pulmonary and arterial pressures in this condition with those of Smith after arsphenamine administration to see that the disturbances are essentially the same in effect.

The final group of experiments, in which repeated doses of arsphenamine were given, shows in addition to agglutinative and embolic phenomena, another type of lesion. This consists

in parenchymatous degenerations of the liver and particularly the kidneys. The degree of these changes varies with the size and number of the doses; in those animals which had received 0.1 gram per kilo on three occasions there was a marked necrosis and calcification of the convoluted tubules of the kidney, while repeated injections of from 0.04 to 0.01 grams per kilo produced only a slight cloudy swelling of the kidney epithelium. This is substantially the result obtained by Kolmer and Lucke.

The question now arises whether or not these parenchymatous lesions may not be the result of vascular obstruction. There is considerable evidence that this is not the case. Although it is true that intravascular agglutination may be demonstrated in those animals which had received the larger doses, 0.1 gram per kilo, when these animals were killed some hours after the injection no tissue lesions were found. A considerable time is required for their development to that stage where the lesion may be recognized morphologically. The same is true of the action on the kidney of such toxic substances as chromium, in which there is no question of a vascular lesion (Ophüls) (25). Moreover it has been shown that embolism of the small vessels of the kidney, such as results from the arterial injection of hardened erythrocytes of another species, produces little if any damage to the organ (Ophüls) (26). Warthin (27) has also shown that in experimental fat embolism the capillary obstruction must be extreme and complicated by stasis and secondary thrombosis before tissue lesions result. When it does occur it is noted in all organs, particularly the brain,[1] whereas in our experiments the liver and kidneys only were definitely involved. And finally the frequent observation in man of extreme necroses in the liver and kidneys following arsphenamine administration without evidence of vascular obstruction, makes it certain we

[1] The brains of many of our animals showed more or less evidence of spontaneous meningo-encephalitis. This had no relation to the arsphenamine administration, being equally marked in animals which died immediately following large doses as in those which had been repeatedly injected with arsphenamine over long periods. A detailed description of this condition which may cause considerable confusion in experiments upon rabbits will appear in a future number of the Journal of Infectious Diseases.

believe, that we have to do here with a factor apart from vascular occlusion.

From our experiments we can therefore differentiate between two types of ill-effects following arsphenamine administration, an early reaction, at times fatal, which is the result of physical factors, multiple embolism of agglutinated red cells, and a late one, in which the predominate finding is one of parenchymatous degeneration of certain organs.

The early reaction is not only due to the physical result of embolism, but, as we have previously shown, the process which results in the formation of the emboli, the agglutination, is also due to factors which are dependent on the physical properties of the arsphenamine solution (28). It is entirely appropriate, therefore, to speak of these early ill effects as the result of the physical toxicity of arsphenamine.

In contrast to this we might speak of the late degenerative lesions as due to the chemical toxicity of the drug, the term "chemical" being used here to indicate that its toxic action depends on its chemical composition rather than on its physical properties. As to the exact mechanism of its action on the protoplasm of the cell, whether by means of chemical or physical processes, our experiments offer no evidence.

If this thesis be admitted, it may still be objected that the physical properties of arsphenamine come into play only when large doses are given. But these physical properties are influenced by many factors. The presence and efficiency of protective colloids, the character and concentration of electrolytes present, the hydrogen ion concentration; all have been shown to effect the process of agglutination in vitro (29). Other experiments have shown that the process of agglutination may be also influenced in vivo. In the body of this paper we have mentioned in another regard some experiments in which sudden death resulted from intravascular agglutination after comparatively small doses. A detailed report on these findings will be given in a later study.

CONCLUSIONS

1. Disodium arsphenamine may produce ill effects by means of either its physical or its chemical properties

2. The *physical* toxicity produces an early reaction and is the result of intravascular agglutination of the red cells and multiple embolism.

3. The *chemical* toxicity requires some time for the production of anatomical lesions. These consist of parenchymatous degenerations particularly in the kidney and liver.

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STUDIES ON STRYCHNIN[1]

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I. THE COMMON GRASS FROG (RANA PIPIENS S̬HREDER) AND THE
QUANTITATIVE ESTIMATION OF STRYCHNIN

Discussion of the literature

It is well known that frogs afford the means of a delicate and characteristic qualitative test for the presence of strychnin in solution, but there is a want of agreement concerning their availability for the quantitative estimation of this alkaloid in extracts of tissues or in nearly pure solution.

von Rautenfeld (1) found that the Rana temporaria (which is one of the two species of frogs most commonly used in Europe in pharmacologic experiments) is unsuited for the quantitative estimation of strychnin. He used seventy-six frogs which varied in weight from 12.5 to 51 grams; to these he administered strychnin in doses varying from 1.463 mgm. per kilogram to 51.612 mgm. per kilogram, and found that death resulted from the largest dose only after the lapse of sixty-nine hours, while the death of the others was entirely irrespective of the dose administered.

R. W. Lovett (2), working under Bowditch, used frogs for the quantitative estimation of strychnin in the cord and other tissues of other frogs which had received very large doses of the poison. He called attention to the discrepancies between the effects on different individuals, which he attributed in part to the "well-known difference in vitality of summer and winter frogs."

Lovett states that Arnold injected 0.000001 gram of strychnin into a frog and observed only slight hyperexcitibility in about half an hour, but that when he injected 0.0001 gram the frog had tetanus from which it recovered. This frog died on the following day from the injection of 0.000001 gram. This statement is so at variance with the results which we have observed and with what one would expect that we would have consulted the original paper of Arnold had it been readily available, but the reference given by Lovett is wrong, and in the paper in which Arnold described his work with extract of nux vomica (in which he used frogs) (3) we have found no statement

such as that cited by Lovett. It hardly seemed worth while to pursue the matter further.

Ipsen (4) states that he believes that the mouse affords an indispensable means of estimating strychnin, in view of the fact that the frog test is valueless, because of seasonal variations in susceptibility.

Ranke (5) calls attention to a difference of dosage of strychnin for frogs which he attributes (inferentially) to a difference between summer and winter frogs.

Hatcher (6) found the fatal dose of strychnin sulphate for the frog (species not recorded) to be 0.45 mgm. per kilogram of weight. Reference to the unpublished protocols of those experiments shows that the results were fairly uniform with a given dose for different individuals. Sollman (7) gives the following doses of strychnin sulphate by hypodermic injection, expressed in milligrams per kilogram of weight; just convulsive, 0.5; just tetanic (leopard frog), 1.0 to 1.5; just fatal, 5.5. Kobert (8) gives the fatal dose of strychnin for frogs (species not stated) as 2.0 mgm. per kilogram of weight. He also states (9) that he cannot agree with Allard and others that the frog is unavailable for the biologic assays of strychnin, but, on the other hand, he has always found it a convenient method of supplementing the chemical reaction.

It is plain from the foregoing brief references to the literature that not all species of frogs are available for the quantitative estimation of strychnin at all seasons, and broad statements sometimes made concerning their use serve to mislead many into the belief that all quantitative estimations of strychnin based on the frog test are unreliable.

While summer and winter frogs, so-called, of some species appear to differ in their reactions toward strychnin, we believe that the results of our experiments throw some light on the cause of such differences, and more particularly on the differences reported in the behavior of individuals of the same species during the course of single investigation carried out during a short period of time.

All of the frogs used by us in the present series of studies were obtained from one dealer in Vermont. Ten of them, taken at random from the tank were identified as Rana pipiens Shreder by Mr. Karl P. Schmidt, of the Department of Taxonomy of the American Museum of Natural History, to whom our thanks are due for this and for other valuable information concerning frogs. He states that the specific name "pipiens" is not commonly recognized in Europe where this frog is usually known as R. virescens.

Mary C. Dickerson (10) states that R. pipiens Shreder is the R. virescens Kalm (Cope) and R. virescens brachycephala Cope. It is easily distinguished from the pickerel frog, the only other common North American frog that resembles it closely, by the fact that the latter has nearly rectangular spots on the back, and orange or yellow colored undersurfaces of the thighs and posterior part of the body.

This frog hibernates during winter at which time few are taken in the northern parts of the United States, hence it would be better to avoid such vague terms as "summer frogs" and "winter frogs."

Storage of frogs

The frogs used during the first of the present series of studies were part of a lot of 500 which we received about November 1, 1920; the remainder were of a lot of 250 received June 18, 1921. The frogs were stored in the manner which has been employed in this laboratory for some ten years past, during which time loss through disease has been negligible.

A soapstone tank, measuring 116 cm. in length, 55 cm. in width, and 19 cm. in depth rests within a larger wooden tank, measuring 180 cm. in length, 68.5 cm. in width, and 29 cm. in depth, from the bottom of which a section has been removed, permitting the top of the soapstone tank to be flush with the floor of the larger one. The wooden tank has a hinged cover which consists of a heavy wire netting, with meshes of 12 mm., attached to a wooden frame. This arrangement permits the frogs to leave the water at will, and it assures free ventilation.

Hydrant water is allowed to trickle into the upper end of the soapstone tank at the rate of about 125 cc. per minute and escape at the opposite end by an outflow that maintains a depth (in the middle of the tank) of 14.5 cm., and a volume of 92.5 liters. This insures the almost complete renewal of the water within a period of about twelve hours, and care is taken to wash the tanks as often as may be necessary, at which time all of the water is allowed to escape.

The temperature of the water in the tank varies less than a degree from that of the inflowing water excepting at such times as the temperature of the air in the room is much above or below that of the water.

Experiments

The frogs designed for an experiment were taken from the water, wiped gently with a towel, and weighed to within 0.1 gram. They were than placed in small wire cages each holding two frogs separated by a partition.

All of the strychnin used was from a single specimen of Merck's strychnin sulphate in crystals, and it was usually administered in freshly prepared solutions containing 1 part of the sulphate in 500,000 parts of normal salt solution.

The injections were made into the ventral lymph sac from a luer syringe, graduated to 0.01 cc., which could be read to within 0.005 cc. Preliminary tests were made to determine whether any difference could be perceived in the activity of the poison dependent upon whether it was injected into the dorsal sac or into the ventral. No difference of this character could be detected.

The injected frogs were returned to their compartments in the wire cages, were wetted thoroughly with water, after which the cages were covered with well wetted towels and kept in the sink until the frogs were to be examined for evidences of hyperexcitability. The cages were then carried to separate tables, or at most two cages were placed on one table, where they were allowed to rest a few minutes before the frogs were examined. We found that a period of diminished reaction to strychnin

followed the removal of the frogs however carefully they were handled, and it was frequently observed that a frog which gave no evidence of hyperexcitability immediately after being placed on the table, gave it distinctly after an interval of two or three minutes. If the frogs made active spontaneous movements at the time that they were examined, they were allowed to become quiet and were then examined after an interval of complete rest. With large doses such precautions were not necessary, but with minimal doses they appear to be essential.

All of the injections were made by one of us and the examinations were made by the other who did not know in any case the size of the dose which had been administered. No one could mistake the sudden violent reflex movements so typical of severe strychnin poisoning, but the only perceptible effect in some cases following minimal effective doses is a slight movement of a single muscle or group of muscles of the thigh or leg, and this is sometimes so slight as to require careful observation for its detection.

Evidences of hyperexcitability were elicited by tapping the cage with the finger, or preferably with a small rubber mallet. The maximum effect of minimal effective doses does not appear usually within less than three hours, hence the frogs were examined after about two hours and a half following the injection, and if the evidences of increased reflex excitability were not unmistakable, the frogs were examined again at intervals during the succeeding half hour. We are unable to say whether this is true of other species of frogs, for we have not seen any reference in the literature to this slow development of the effects of small doses. It is especially noteworthy in view of the well-known fact that convulsions are commonly observed within a few minutes after the injection of strychnin in doses only slightly greater than the minimal required to induce them.

The minimal effective dose of strychnin sulphate that caused unmistakable increased reflex excitability in the frog was found to be equal to 0.15 mgm. per kilogram of weight. It will be seen that an occasional irregularity occurs in the dose required, but this is only what one would expect, and we have performed a

sufficient number of experiments in each series to render gross error impossible. Normal frogs were used as controls in the earlier experiments, and the one who examined them was not informed at the time which had had strychnin.

We could not fix the minimal effective dose of the tissue extracts with precision in every case, without the expenditure of more time, and the use of a greater number of frogs, than the results seemed to justify, in view of the fact that the error was never such as to affect the conclusions materially.

In some of the experiments it was apparent that the lowest dose administered which caused perceptible effects was actually very near to the minimal, and in such cases it was accepted as the minimal, but in others the lowest effective dose actually administered caused marked hyperexcitability, and in those tests we accepted the mean between the lowest effective, and the highest ineffective, doses as representing the minimal, that is, as corresponding to doses of 0.15 mgm. of strychnin sulphate per kilogram of weight. The following example illustrates the method of estimating the amount of strychnin present in such cases.

A dose of 0.3 cc. caused hyperexcitability in a frog weighing 31.3 grams, and all larger doses were effective; 0.2 cc. failed to induce perceptible effects in a frog of 30.2 grams, and a smaller dose was also ineffective. Total extract to be tested measured 40 cc. The amount of strychnin present is calculated in the following way:

$$0.3 : 40.0 :: 31.3 : 4173; \text{ and } 0.2 : 40.0 :: 30.2 : 6040.$$

The larger dose indicates the presence of 0.62 mgm., or enough to cause increased reflex excitability in frogs weighing a total of 4173 grams; $(4.173 \times 0.15 = 0.626)$; the result of the smaller dose being negative, it indicates the pressure of less than 0.9 mgm. or less than enough to cause hyperexcitability in frogs weighing a total of 6040 grams; $(6.04 \times 0.15 = 0.90)$; the mean of these two (0.62 mgm. and 0.9 mgm.) is 0.76 mgm., and this is accepted as the amount present.

There are many factors that influence the action of strychnin in the frog but we were concerned primarily with the availa-

bility of the common grass frog (R. pipiens Shreder) for the quantitative estimation of strychnin in a series of studies that we had planned, and we have therefore investigated only those

TABLE 1

Showing the minimal effective dose of crystalline strychnin sulphate required to cause perceptible increased reflex excitability in the grass frog (Rana pipiens Shreder) after prolonged fasting

WEIGHT	DOSE PER KILOGRAM	RESULT
grams	*mgm.*	
31.5	0.20	Positive
56.0	0.19	Positive
41.3	0.18	Positive
49.0	0.17	Positive
38.4	0.16	Positive
54.0	0.15	Positive
34.2	0.15	Positive
32.9	0.15	Positive
28.4	0.15	Positive
68.0	0.15	Positive
68.3	0.15	Positive
25.7	0.14	Negative
60.0	0.13	Negative
44.0	0.12	Negative
57.8	0.12	?
41.0	0.12	?
25.0	0.12	Positive
30.6	0.12	?
35.1	0.11	Negative
42.4	0.10	Negative
24.7	0.10	?
35.7	0.10	Negative
38.8	0.05	Negative
35.8	0.05	Negative
40.1	0.05	Negative
48.8	0.02	Negative

Controls all negative.

problems which seemed to us of importance in our investigations. Without pursuing the several lines of investigation exhaustively we have studied the influence of variations in weight and in the seat of the injection; the influence of fasting, removal of the liver, and of keeping frogs in dry air or in water.   We have also sought

to determine whether frogs that had been used recently for the quantitative estimation of strychnin could be used again for this purpose, and also whether the intensity of the action of strychnin is fairly constant for frogs of a single lot, as well as for those of different lots, during a period of some months with changes of temperature incidental to winter, spring and summer.

Influence of weight on the action of strychnin

Directions for the use of frogs for the quantitative estimation of drugs or poisons usually state categorically, or by inference, that only slight differences in the weights of the individuals employed are permissible.

An approach at uniformity of weight involves the rejection of many frogs, and if the effective dose of strychnin per gram does vary materially with the weight, we should know the limits of permissible variation.

Fourteen frogs, varying in weight from 14.2 grams to 60.6 grams, were injected in pairs with doses varying from 0.55 mgm. per kilogram (the convulsive dose) to 0.15 mgm per kilogram. No differences were observed in the intensity of action that could be attributed to the difference in weight, and while we did not determine the exact interval following the injection before the onset of symptoms, we can say that differences in weight caused no gross differences in this interval.

Influence of moisture on the toxicity of strychnin

Frogs lose weight in dry air through evaporation of water, and it seemed to us that this might exert an important influence on the rate of the absorption of strychnin, and in that way influence the intensity of the action. Twenty-six frogs were used in the experiments designed to show whether any influence could be attributed to differences of this nature.

Doses varying from 0.15 mgm. per kilogram to 0.05 mgm. per kilogram were administered. All of those (6) which received the larger dose showed hyperexcitability of about the same degree; fifteen of those which received less than that dose (and

including all of those that were then kept in dry air, 6) showed no perceptible effect; the remaining five were recorded as showing slight increased reflex excitability, but the results with these five were so irregular that we are inclined to think that an error was made in at least two or three of these.

These were the first experiments in which very small doses were used and we believe that we mistook a voluntary movement, or an involuntary one caused by shaking the cage, for the evidences of increased reflex excitability, but there is no question that slight increased reflex excitability does occasionally result from a dose of less than 0.15 mgm. per kilogram. This occurred only seldom in subsequent experiments, hence our belief that not all of the five actually showed the effects of strychnin.

Since there is a possibility that the intensity of the action of strychnin may be lessened by keeping the injected frogs in dry air we adopted the practice of keeping them in moist.

Availability of previously used frogs

The advantages of being able to use experimental animals repeatedly are obvious and do not call for discussion. We have occasionally seen frogs remain extended for a period of more than a week after the injection of a single dose of strychnin, and during that time a slight stimulus sufficed to cause tetanus. More frequently they appear normal within twenty-four to forty-eight hours after having had strychnin in doses just sufficient to cause convulsions.

The frogs that were used in the earlier experiments were placed in a tank in which the water was renewed frequently. Eight of these which had not had strychnin within a period of less than four weeks were selected for the following experiment. Strychnin sulphate was administered to all of these in doses of 0.75 mgm. per kilogram; they showed tetanus in about an hour; markedly increased reflex excitability on the following morning, and some in the afternoon. They were examined several times during the succeeding forenoon without evidence of hyperexcitability being observed. During the afternoon (about 48 hours

after the previous dose of 0.75 mgm. per kilogram) two of them received injections of 0.1 mgm. per kilogram, and became nearly convulsive; two that received doses of 0.05 mgm. per kilogram showed about as much effect as those that received the larger doses, while three that received doses of 0.05 mgm. per kilogram showed only slightly increased reflexes, and one that received a dose of 0.04 mgm. per kilogram showed no perceptible effect.

It is of some interest to observe that five of the eight frogs showed distinctly increased reflex excitability from doses only one third as large as those required by normal (unused) frogs to induce this effect, and that the effect was much greater in these previously used frogs than with three times as much in normal frogs.

The results of this experiment show that frogs may become normal in appearance after receiving convulsive doses of strychnin without actually becoming so with reference to their reaction toward minimal doses of that alkaloid, and this is to be expected in the period immediately following the cessation of increased reflexes, because the animals are then in the same condition, presumably, that they would be if they had just had doses slightly under those required to cause perceptible effects.

Four frogs that had received small doses of strychnin during the previous week (none within six days) were given doses of 0.15 mgm. per kilogram—doses which would just suffice to cause increased reflexes in normal frogs. One became convulsive on slight stimulation, the other three showed some increased reflex excitability.

Eight of the previously used frogs, which, however, had received no strychnin within a period of four weeks, were injected with doses varying from 0.17 mgm. per kilogram to 0.1 mgm. per kilogram. One of two that received doses of 0.1 mgm. per kilogram showed no effect; the other one that received this dose became nearly convulsive on stimulation. Six that received the larger doses showed varying degrees of increased reflexes.

Six of the same lot of frogs (used at least four weeks previously) were given doses of 0.15 mgm. per kilogram. Three became

nearly convulsive on stimulation, the others showed moderately increased reflexes.

It is remarkable that frogs recover so far as to lose their increased reflex excitability within forty-eight hours after the administration of strychnin in doses of 0.75 mgm. per kilogram, but fail to recover completely within a period of four weeks after the administration of much smaller doses.   One may theorize that the poison is not actually eliminated or destroyed, but that the reflex mechanism becomes accustomed to its effects, but while the question has an interest far greater than that of the repeated use of the same frogs for the quantitative estimation of strychnin, we have been unable to pursue the investigation further. We have not used frogs a second time in any of the other experiments excepting those dealing with the question of the influence of fasting.

It was essential to know whether frogs vary quantitatively from month to month in their reaction toward the standard dose of strychnin which we adopted, i. e. that which is just sufficient to cause perceptible increased reflex excitability, and if so, to what degree.   We have, therefore, investigated this question.

The minimal dose of our specimen of strychnin sulphate was determined on February 24, 1921, with frogs that had been stored since about November 1, 1920, as previously stated. At that time it was found that this dose is equal to 0.15 mgm. per kilogram of weight.   The dose was determined again on April 16, and again on June 11, with frogs of the same lot and no difference in the amount required could be detected.

The influence of fasting

We received a fresh lot of frogs on June 18.   Eighteen of these were used four days later for the determination of the standard dose.   Thirteen of these received doses varying from 0.2 mgm. per kilogram to 0.1 mgm. per kilogram without showing any perceptible effect; two received doses of 0.35 mgm. per kilogram and 0.4 mgm. per kilogram respectively without showing any effect; while the results with three others, which received doses of 0.25 mgm., 0.28 mgm., and 0.3 mgm. per kilo-

gram were doubtful, though almost certainly negative. Seven frogs of this lot were examined on July 1, (fourteen days after having been received). One showed no effect from a dose of 0.1 mgm. per kilogram; one that received a dose of 0.15 mgm. per kilogram showed a possible effect, while those that received doses of 0.2 mgm., 0.25 mgm., 0.3 mgm. and 0.35 mgm. per kilogram showed distinct hyperexcitability, and one that received 0.4 mgm. per kilogram was almost convulsive on stimulation. The results of this experiments indicated a distinct increased average susceptibility. Tests were made on July 5 and July 11. The frogs behaved on July 5 almost exactly like those of the previous lot, and when examined on July 11, no difference could be perceived between these frogs and those that had been on hand since the previous November.

While there could be little doubt that the increasing susceptibility of these frogs was due to fasting, we undertook to secure evidence in support of this view by feeding frogs that had fasted for eight months, and comparing their susceptibility to strychnin with that of others of the same lot that continued to fast. All of these (those that were fed and the controls) had been used previously, but none had received strychnin during a period of about two months.

Ten frogs were placed in a cage in water and about 1 gram of chopped raw beef's liver was fed to each on alternate days—July 5, 7, 9—after which nine of these (one having died) and eight of the same lot which had continued to fast, and which were used as controls, were injected with strychnin on July 11. Feces were found in the cage and in the intestines of the frogs that were fed, while the intestines of those that served as controls were found to be empty.

The table shows the weights of the frogs, the doses expressed in fractions of a milligram of strychnin per kilogram of weight, and the effects.

The contrast shown between the results obtained with the fasting frogs and those with the frogs that were fed, leaves little to be said concerning the effects of fasting on the susceptibility of frogs toward small doses of strychnin, but we have tried to throw some light on the cause of this.

TABLE 2

Showing increasing suceptibility of the grass frog toward strychnin sulphate during the first weeks of fasting.   All of these frogs were received on June 18, 1921

WEIGHT	DOSE PER KILOGRAM	RESULT
June 22		
grams	*mgm.*	
32.0	0.40	Negative
31.2	0.30	Negative
40.0	0.28	Doubtful*
44.0	0.25	Doubtful*
54.5	0.20	Doubtful*
31.7	0.20	Negative
34.3	0.20	Negative
33.6	0.16	Negative
29.0	0.16	Negative
42.2	0.15	Negative
51.5	0.15	Negative
38.0	0.14	Negative
38.7	0.14	Negative
39.6	0.12	Negative
41.0	0.12	Negative
44.0	0.10	Negative
46.5	0.10	Negative
June 23		
44.0	0.30	Doubtful
39.5	0.30	Positive
July 1		
23.5	0.40	Alm. conv.
25.0	0.35	Positive
31.5	0.30	Positive
28.0	0.25	Positive
38.0	0.20	Positive
30.3	0.15	Doubtful
33.8	0.10	Negative
July 5		
26.5	0.20	Positive
31.8	0.18	Doubtful
30.5	0.15	Positive
31.4	0.13	Positive
33.0	0.10	Negative

TABLE 2—*Concluded*

WEIGHT	DOSE PER KILOGRAM	RESULT
July 11		
grams	*mgm.*	
29.5	0.20	Positive
32.2	0.15	Positive
34.2	0.13	Negative
33.0	0.10	Negative

* Recorded as doubtful but almost certainly negative, the reflexes being elicited only when cage was struck with some violence.

TABLE 3

Showing the comparative reaction of fasting frogs and those which had been fed after prolonged fasting*

WEIGHT	DOSE PER KILOGRAM	RESULT
Frogs that had been fed		
grams	*mgm.*	
48.8	0.10	Negative
49.0	0.15	Negative
35.2	0.15	Negative
37.0	0.20	Negative
37.2	0.25	Positive
32.5	0.30	Negative
27.0	0.35	Negative
27.0	0.40	Doubtful
21.5	0.45	Positive
Frogs that continued to fast		
46.0	0.10	Doubtful
32.5	0.15	Positive
38.5	0.20	Positive
47.0	0.25	Positive
30.0	0.30	Positive
28.4	0.35	Positive
26.0	0.40	Positive
23.2	0.45	Tetanus

* All of the frogs used in these experiments had been used previously but the interval permitted of almost complete recovery from the effects of the poison.

The rôle of the liver

Strychnin is destroyed in the bodies of animals of several species, including the cat, dog, and guinea pig, following its administration, and in the liver during perfusion of that organ with a solution containing the poison (11) and it seemed probable that the liver of the frog is concerned with its destruction.

The livers of the frogs that had fasted for some months were of a dark grey color, and little or no bleeding occurred when the organ was deeply incised. Fatty degeneration was observed in several. The liver of the recently fed frog is much redder and bleeds freely when cut. The liver of the frog that has fasted for a prolonged period can have little effect on the injected strychnin if the blood has practically ceased to circulate through that organ, and one would anticipate that its removal would then have little influence on the dose of strychnin required to cause increased reflex excitability, whereas the removal of the liver of the recently fed frog would deprive the animal of its protective mechanism, and thereby increase the susceptibility of the animal to strychnin. The tabulated results of the following experiment show that this is the fact.

The livers were removed from ten frogs that had been received five days previously, and from four frogs that had fasted during a period of some months.[2]

Twenty-four hours were allowed to elapse in order that the frogs might recover from the effects of the operation, after which they were injected with strychnin in doses varying from 0.1 to 0.3 mgm. per kilogram of weight, and two of the normal frogs, which had been received six days previously, received doses of 0.30 mgm. per kilogram each in order that they might serve as controls. These two were far more resistant to the poison than were any of those from which the liver had been removed. All of those which had been deprived of the liver and those which

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[2] The liver was removed through an incision made to the right of the median line in order to avoid the large vein in that region; a ligature was tied around the base of the liver, the tissue was cut above the ligature, the liver was removed practically without the loss of blood (other than that in the liver), and the incision was closed. There was hemorrhage in one case and that frog died.

had fasted during some months showed about an equal degree of susceptibility toward strychnin.

The results of this experiment afford evidence that the liver of the frog plays an important rôle in the destruction or elimination of strychnin following its injection during periods of active

TABLE 4

Showing the susceptibility toward strychnin of frogs after removal of the liver

WEIGHT	DOSE PER KILOGRAM	RESULT
After fasting for some months		
grams	*mgm*	
55.9	0.10	Negative*
49.5	0.15	Positive
48.2	0.20	Positive
40.4	0.25	Positive
Received six days previously		
31.0	0.10	Positive slight
48.5	0.10	Positive slight
47.0	0.15	Positive
47.6	0.15	Positive
43.2	0.20	Positive marked
42.3	0.20	Positive marked
36.0	0.25	Positive marked
34.3	0.25	Positive marked
35.0	0.30	Nearly convulsive
31.8	0.30	Nearly convulsive
Normal frogs, received six days previously		
39.5	0.30	Positive slight
44.0	0.30	Doubtful

* Negative after three hours, became slightly positive after four hours.

metabolism, but that it does not when the poison is administered after a period of prolonged fasting. Frogs which are selected for use in the quantitative estimation of strychnin should be fed uniformly or care should be exercised that they do not obtain food, such as flies or other insects. Failure to observe such precautions may result in variations in susceptibility of 400 per cent, and possibly in even greater variations,

for we have no data concerning the susceptibility of the frog which has suffered no interruption in its normal food supply.

It seems to us that it is more than possible that some of the results obtained by other observers who have reported that the frog is unsuitable for the quantitative estimation of strychnin, may be explained in part by the fact that such precautions were not observed, but it does not seem probable that the extraordinary variations in the reaction of Rana temporaria toward strychnin which von Rautenfeld reported, can be explained in this way.

We do not maintain that every lot of frogs of this species—Rana pipiens Shreder—will behave exactly as ours did, even when similar precautions are taken, but we do believe that no one is justified in concluding that the frog is unsuited for use in this way if no account is taken of the factors that we have discussed. It may be stated in passing that one of us (H) has found several lots of frogs obtained from the dealer who supplied those with which this work was done, wholly unsuited for the bio-assay of digitalis because of extraordinary differences in the capacity of different individuals to absorb that drug. We have been informed orally by several others that they have experienced similar trouble in the bio-assay of digitalis, while others who obtained their frogs from western dealers reported that they had had no such difficulty.

Summary

1. The common grass frog, or leopard frog (Rana pipiens Shreder) obtained from Vermont reacts with a near approach to uniformity to small doses of strychnin so that it can be used after a period of fasting until the metabolism is reduced to the minimum for the quantitative estimation of strychnin in nearly pure solution.

2. A dose of crystalline strychnin sulphate (Merck's) equal to 0.15 mgm. per kilogram of weight suffices to cause perceptible increased reflex excitability in the grass frog after it has fasted until metabolism has become minimal. This period of fasting probably varies, but under the conditions described in this paper it is about three or four weeks.

3. Frogs which have been recently caught and in which metabolism is active also react nearly uniformly toward small doses of strychnin but the amounts then required by them to induce increased reflex excitability are several times greater relative to the weight than those required by frogs of the same lot after they have fasted until metabolism is minimal.

4. The tolerance of recently caught (and fed) frogs toward strychnin diminishes gradually, and apparently, irregularly.

5. Grass frogs which have fasted until their tolerance is minimal, may have their tolerance toward strychnin increased by suitable feeding. It is important, therefore, that frogs which are used for the quantitative estimation of strychnin shall be fed uniformly or that they shall be deprived of food, including insects.

6. The liver of the frog is concerned in the destruction or elimination of strychnin when the poison is administered during the period of active metabolism, but not when it is administered during a period of minimal metabolism, that is to say, after a long period of fasting.

7. The removal of the liver of the frog during a period of minimal metabolism (after long fasting) has little influence on the size of the dose of strychnin required to induce increased reflex excitability. The removal of the liver during active metabolism causes an increase in its susceptibility toward small doses of strychnin so that it then behaves like a frog which had fasted for a long period, or until its metabolism was minimal.

8. The frog having a minimal metabolism becomes apparently normal within a period of forty-eight hours after the injection of a dose of strychnin which is just sufficient to cause tetanus, so that no increased reflex excitability can be detected after the lapse of such a period, but there is a certain degree of latent persistent action, since such frogs exhibit an increased susceptibilty toward strychnin for periods of several weeks after the administration of much smaller doses than those from which they apparently recover within forty-eight hours. We are unable to offer any satisfactory explanation of this phenomenon.

9. It is immaterial, so far as the intensity of the reaction is concerned, whether the strychnin is injected into the dorsal, or into the ventral, lymph sac.

10. Variations in the weights of the animals within wide limits are without influence on the amount of strychnin per gram of weight required to cause increased reflex excitability.

11. The terms "summer frogs" and "winter frogs" as they are commonly used, are vague and tend to mislead. It is desirable that those who use frogs for the quantitative estimation of strychnin state the species employed and the conditions under which they are stored previous to use.

II. THE EXTRACTION OF STRYCHNIN FROM TISSUES

In pursuance of our plan to study the rate of disappearance of strychnin from the circulating blood we employed the method (somewhat modified) which Hatcher and Eggleston (11) had found satisfactory for the recovery of the alkaloid after they had added it to defibrinated blood in amounts varying from 10 to 100 mgm. The results of our preliminary experiments showed that this method is not satisfactory when one is dealing with less than a milligram of strychnin in the blood of a cat of average size.

Great interest was aroused in the problem of recovering strychnin from tissues in consequence of the celebrated trial of Palmer, who was accused of the murder of Cook in 1855. Most of the resulting literature has been forgotten, but one still sees an occasional reference to some of the statements then made concerning the ease and certainty with which strychnin can be detected in the body when only traces are present at the time of death.

Analysis of some early work

We are not aware that any analysis has been made of the evidence—or, more accurately, the total lack of it — on which many of these statements were based, though arguments, equally faulty, were common. Two statements which were widely accepted will be examined briefly.

Taylor, the chemist, failed to find strychnin in the gastric contents which had apparently become contaminated with fecal matter and blood and he was subjected to severe criticism by Rodgers and Girdwood (12) because of the methods that he used.

Rodgers and Girdwood poisoned a rabbit (13) with large doses of strychnin, the animal died about half an hour after the administration of the last dose of one-sixth of a grain, and they recovered some of the poison from the stomach and tissues of the animal. They state that strychnin can always be found in the tissues of an animal poisoned with it, even when it is not present in the stomach. They also say that the delicacy of the reaction and the extraordinary stability of the alkaloid make it the most easily detected of poisons. They speak of 1/2000 grain as a relatively large amount when discussing the chemical test for it in pure solution, plainly implying that even that amount could be detected if it were present in the animal body. They record no experiments in which they detected strychnin when only traces were added to tissues or administered to an animal.

De Vrys (or De Vrij) and Van der Burg (14) evaporated one drop of a solution containing 1/60,000 grain of strychnin on a porce'ain plate, and were able to observe the purple color caused by adding concentrated sulphuric acid and a small crystal of potassium bichromate to the residue on the plate.

They were able to recover strychnin almost quantitatively after adding it to the white and yolk of an egg, to six ounces of meat, and to a specimen of urine in amounts of one-fourth of a grain in each case. They also poisoned a dog with half a grain of strychnin and found an abundance of the poison in the stomach, but they could not detect a trace of it in the tissues of the animal.

Since they could detect so little as 1/60,000 of a grain (in pure solution), and since they could recover it almost quantitatively when added to tissues in amounts of a quarter of a grain, they maintained that they could also recover it from the body when only traces were present, but they record no experiments in which they even attempted to recover strychnin after

adding traces of it to tissues, but, on the contrary, they failed to detect it in several of their experiments when it was unquestionably present in relatively large amounts.

It is certain that the method used by Rodgers and Girdwood and that used by De Vrys and Van der Burg were faulty and do not permit of the detection of strychnin when only traces are present in the body of an animal, such as the dog, and we know of no experiments except our own in which traces of strychnin have been added to relatively large amounts of tissue and recovered almost quantitatively. We do not wish to have it understood, however, that we can detect strychnin when no more than a fraction of a milligram is distributed throughout the human body, but we have some evidence that tends to show that we could detect this poison in the blood of a human adult if as much as 0.5 mgm. were present.

One cannot appreciate the full significance of this question without some knowledge of the behavior of strychnin in the body, for it must happen occasionally that the detection of the merest trace of the poison, when taken with other circumstantial evidence, will suffice to fix a crime upon the guilty, and we shall therefore review briefly the chief points of interest.

Strychnin is usually absorbed fairly rapidly after its oral administration, and when death is delayed for an hour or more there may be none present in the stomach or duodenum when these are examined. The poison begins to appear in the urine within a few minutes after it enters the circulation, but the total amount that is excreted by the kidney varies widely in different cases. Strychnin is certainly destroyed in part in the bodies of many animals, and almost certainly in the liver, and when death occurs some hours after the ingestion of the poison there may be only traces of it in the body. Owing to the importance of this question we have extended our investigation of the matter further than was demanded by the original purpose in view. The experiments are not presented in the exact order in which they were performed.

The first of the experiments was designed to show whether we could recover nearly all of a very small amount of strychnin after adding it to blood.

One milligram of strychnin sulphate, dissolved in 1 cc. of normal salt solution, was added to 119 c.c. of strained defibrinated blood of a cat; the mixture was divided into two portions, one of which was extracted and the other reserved, but not subsequently used. Sixty cubic centimeters of the mixture, containing 0.5 mgm. of strychnin sulphate, was shaken with enough alcohol to make the mixture measure 250 cc.; the mixture was allowed to stand until the clear supernatant liquid, equal to half of the volume, could be decanted; this was evaporated on a water bath; the residue was taken up in water to which a little tartaric acid had been added; the solution was transferred to a separatory funnel and rendered alkaline by the addition of sodium hydrate, after which it was shaken with successive portions of chloroform until extraction was virtually complete; the chloroform was distilled; the residue, weighing 8 mgm., was dissolved in 8 cc. of normal salt solution containing a trace of sulphuric acid. The amount of strychnin sulphate present in this 8 cc. of solution, representing the extract of one fourth of the total blood to which strychnin had been added, was estimated by testing it on frogs in the manner described in the previous paper.

Tests showing the amount of strychnin extracted from tissues by means of alcohol

WEIGHT	DOSES OF EXTRACT, TOTAL	RESULT
grams	cc.	
9.5	1.00	Tetanus
14.7	0.80	Tetanus
10.0	0.50	Positive
10.7	0.30	Positive
10.3	0.20	Positive
13.0	0.15	Negative

See text for significance of these results.

The tabulated results of this test indicate that we recovered more than 25 per cent, less than 40 per cent, of the strychnin that was added to the blood represented by the extract. This estimate is based on the following calculation: Since 0.2 cc. of the solution caused hyperexcitability in a frog weighing 10.3

grams, 8 cc. would cause hyperexcitability in at least 412 grams of frogs, indicating the recovery of (0.412 kgm. × 0.15 mgm. =) 0.062 mgm. of strychnin sulphate, or 25 per cent of that added to the portion of the blood represented by this extract. Since 0.15 cc. failed to induce any perceptible effect in a frog weighing 13.0 grams, 8 cc. would cause no perceptible effect in 694 grams of frogs, indicating the recovery of less than (0.694 kgm. × 0.15 mgm. =) 0.1 mgm., or 40 per cent of the strychnin added to one-fourth of the blood.

Strychnin sulphate is quite soluble in diluted alcohol, but it seemed possible that the failure to recover a larger percentage of the strychnin in this experiment might be attributed to our failure to use acid in the extraction of the blood, the next experiment was therefore carried out in the following manner:

Extraction by modified Stas-Otto method

One milligram of strychnin sulphate was added to 72 cc. of strained defibrinated blood of a cat, the mixture was acidulated with tartaric acid and heated gently, after which enough alcohol was added to make the volume 500 cc.; this was triturated thoroughly and the mixture allowed to stand over night. The liquid was then strained, and 415 cc., representing 83 per cent of the whole, was evaporated; the residue was taken up in water; the solution was transferred to a separatory funnel, rendered alkaline by the addition of sodium hydate, and shaken four times with successive portions of chloroform, the extraction being virtually complete. The several portions of chloroform were washed with water, after which the mixed chloroform was distilled, and the residue, weighing 5 mgm., was dissolved in 15 cc. of normal salt solution containing a trace of sulphuric acid.

This solution was tested on frogs in the usual manner, the results indicating the recovery of more than 17.5 per cent, and less than 36 per cent, of the strychnin sulphate that had been added to this fraction (83 per cent) of the blood.

These results showed that this method of extraction was unsuited for the investigation that we had planned, and we undertook to extract the strychnin in the following manner.

Extraction with chloroform after liquefying tissues with sodium hydrate

One milligram of strychnin sulphate in solution was added to 100 cc. of whole blood obtained from a cat; this was heated on a water bath after the addition of 10 cc. of 30 per cent solution of sodium hydrate, until complete solution was obtained; the mixture was transferred to a shallow porcelain dish together with the washings of the vessel in which it was heated, and an equal volume of chloroform was added, after which the mixture was rotated gently for half an hour. The chloroform was separated, and the mixture was treated with twice the volume of chloroform in each of two successive portions for periods of an hour in each case. A fresh portion of 4 volumes of chloroform was then added the dish and the whole allowed to stand over night. The mixture above the chloroform formed a layer about 5 mm. in thickness, being about as thin as the surface tension permitted.

The several portions of chloroform were washed with water, after which they were mixed and the chloroform was distilled; the residue, weighing 8 mgm., was dissolved in 50 cc. of normal salt solution containing a trace of sulphuric acid, and the amount of strychnin sulphate present was estimated by testing the solution on frogs in the usual way. The tabulated results indicate the recovery of at least 92 per cent, less than 108 per cent, of the strychnin that was added to the blood. The hyperexcitability shown by frog No. 11 was minimal, and we may accept this as indicating the actual amount of strychnin recovered. (See table on next page.)

Having found that we could recover nearly all of 1 mgm. of strychnin sulphate in this way, we undertook to determine whether we could detect evidences of its presence in amounts corresponding to 0.5 mgm. of the poison in 5 liters of blood, the approximate volume of that of the average adult human being. Unpoisoned blood was extracted at the same time, and the extract tested on a frog in order to show that normal blood yields nothing capable of causing hyperexcitability in the frog when extracted in this way. The protocol of the experiment is given in brief.

A solution of 0.01 mgm. of strychnin sulphate was added to 110 cc. of whole blood of the cat, the mixture was shaken and allowed to stand for a few minutes, after which 15 cc. of 30 per cent solution of sodium hydrate were added, and the mixture heated on a water bath until solution was completed, the mixture was then cooled and treated with chloroform in the manner just described for the extraction of 1 mgm. of strychnin sulphate. The residue left after the distillation of the chloroform, weighing 7.4 mgm., was treated with concentrated sulphuric

Tests showing the amount of strychnin recovered by extraction with chloroform after liquefying tissues with sodium hydrate

NUMBER OF EXPERIMENT	WEIGHT	DOSE OF EXTRACT	RESULT
	grams	*cc.*	
1	48.0	1.00	Positive
2	43.5	0.80	Positive
3	38.2	0.65	Positive
4	36.0	0.50	Positive
5	34.0	0.40	Negative
6	33.0	0.35	Positive
7	30.8	0.31	Positive
8	29.0	0.28	Positive
9	28.0	0.26	Positive
10	28.0	0.24	Positive
11	27.6	0.22	Positive
12	25.0	0.18	Negative

Since 0.22 cc. of the extract caused minimal hyperexcitability in a frog weighing 27.6 grams, 50 cc. of the extract would cause hyperexcitability in frogs weighing in the aggregate 6.26 kgm. This would require 0.92 mgm. of strychnin sulphate. The result with no. 5 is clearly out of order and is therefore disregarded.

acid in order to destroy organic matter, as suggested by Rodgers and Girdwood (12) after which the residue was taken up in water, rendered alkaline by the addition of sodium hydrate, and the mixture shaken with chloroform in several successive portions. The residue left after distilling the chloroform was taken up in a few drops of normal salt solution, containing a trace of acid, and the whole of this solution A. was injected into a frog weighing 15 grams, a drop or two of normal salt solution was then used to wash the watch glass and syringe and this was injected into the frog.

The frog showed marked hyperexcitability within about an hour, so that a nearly spasmodic condition was induced when the cage was tapped rather sharply. A delayed return to the normal position seemed to indicate that the blood may yield traces of some substance that has some effect on frogs.

Another portion of blood taken from another cat was treated in the manner just described except for the fact that normal salt solution was added instead of solution of strychnin sulphate. The solution of the repurified extract was injected into a frog weighing 23 grams, but no perceptible effect was induced.

The solutions of the extracts were labeled A and B and the one who made the injections did not know which represented the poisoned blood, and the frogs were examined by one of us who did not know which frog had received the solution of the extract of the poisoned blood, but these precautions proved unnecessary, for the frog which received this showed such a degree of increased reflex excitability that there was no possibility of a mistake by the least skilled observer.

The results of this experiment left no doubt in our minds that we could detect as little as 0.5 mgm. of strychnin when present in 5 liters of blood, and in fact, 0.25 mgm. could almost certainly be detected, for the extract of the blood to which 0.01 mgm. had been added, was more than the minimal required to cause hyperexcitability in this frog, and the purification of minute amounts of solution presents difficulties that are much less when one deals with a relatively large amount.

The Stas-Otto method, variously modified, for the extraction of tissues is widely used and we wished to learn whether it suffices for the estimation of very small amounts of strychnin in other tissues and to compare the results obtained with it with those gotten after the tissues are dissolved in sodium hydrate and the extraction with chloroform is then carried out in the manner described. The protocol in brief of an experiment is given in order to show the result of such an extraction of muscle.

Two hundred grams of muscles from the thighs of an exsanguinated cat were divided into two similar portions which were labeled A and B, respectively. One milligram of strychnin

sulphate dissolved in 10,000 parts of normal salt solution was injected into each portion of the muscle so that the solution was distributed as uniformly as possible. After an interval of an hour each portion was hashed separately in a machine, which was washed with water, the water being added to the hashed muscle in each case. The two portions were then extracted as follows:

A. Fifty cubic centimeters of 30 per cent sodium hydrate solution were added to the hashed muscle and the mixture was heated in a porcelain dish until solution was effected; the solution was evaporated in a current of air over boiling water until the volume was reduced to 150 cc., after which the solution was extracted with chloroform in the manner already described.

The residue left after the distillation of the chloroform weighed about 0.5 gram and consisted largely of soap; this was treated with very dilute sulphuric acid, the solution was transferred to a separatory funnel, rendered alkaline by the addition of sodium hydrate solution, and extracted with chloroform. The residue left after the distillation of the chloroform, weighing 5 mgm., was dissolved in 35 cc. of normal salt solution containing a trace of sulphuric acid, and the solution was tested on frogs in the usual way.

B. The second portion of 100 grams of muscle and the washings of the hashing machine were shaken with 300 cc. of alcohol which had been acidulated slightly with tartaric acid, after which a few drops of 10 per cent aqueous solution of tartaric acid were added in order to render the mixture slightly, but distinctly, acid. The mixture was boiled for fifteen minutes in a flask connected with a reflux condenser with frequent shaking; it was then cooled and filtered through a soft filter having a diameter of 20 centimeters. The residue on the filter was washed with 50 cc. of acidulated alcohol, and the mixed filtrates were evaporated on a water bath to a syrupy consistence. To this were added 100 cc. of cold water, which caused a copious precipitate. The mixture was filtered through a soft filter paper of 16 cm. diameter, and the residue washed with 25 cc. of water, after which the mixed filtrates were evaporated on a water bath

and the residue was almost completely dissolved in 50 cc. of water; this solution was filtered, and the filtrate, after having been transferred to a separatory funnel, was rendered alkaline by the addition of sodium hydrate; extraction with chloroform was then carried out in the same way as with the first portion.

The residue, left after the distillation of the chloroform, weighing 5 mgm., was dissolved in 20 cc. of normal salt solution containing a trace of sulphuric acid, and this was tested in the usual way.

The residue of the muscle left on the filter after extraction and washing with acidulated alcohol (B) was partially dried and the residue dissolved with the aid of 15 cc. of 30 per cent solution of sodium hydrate and the solution was extracted with chloroform in the same way that A was extracted. The residue, left after the distillation of the chloroform, weighing 8 mgm., was dissolved in 5 cc. of normal salt solution containing a trace of acid, and this solution was tested on frogs.

The tabulated results indicate that the extract of A, made by dissolving the muscles in alkali and extracting directly with chloroform, contained 0.79 mgm. of strychnin sulphate; that B, made by extracting with acidulated alcohol, contained about 0.18 mgm., and that the extract of the residue of the muscle left after the extraction with acidulated alcohol, (C) contained rather more than 0.19 mgm. The total extracted in B and C, 0.37 mgm., was much less than that extracted in A.

The failure to recover greater percentages of small amounts of strychnin which had been added to tissues in those experiments in which acidulated alcohol was used for the extraction is explained in part by the retention of the poison by the insoluble tissue, but more than half of the loss occurs elsewhere.

Another experiment was performed in the manner just described except for the fact that all residues and filter papers (employed in the extraction of B) were used for the preparation of the extract called C. The results of the tests on frogs in this experiment indicated that the A fraction (direct extraction with chloroform after dissolving the tissue in sodium hydrate solution) contained 0.75 mgm.; that the B. fraction (extraction

with acidulated alcohol) contained 0.22 mgm.; and that the
C. fraction (all residues) contained 0.5 mgm.   It is interesting
to observe that the total in B and C—0.72 mgm.—is almost
exactly the same as that in A—0.75 mgm.

*Tests showing strychnin extracted from muscle by means of: A, Extraction
with chloroform after liquefying muscle with sodium hydrate. B, With acidu-
lated alcohol (modified Stas-Otto method). C, Extraction by applying method
A to residues of muscle left after extraction with acidulated alcohol*

WEIGHT	TOTAL	RESULT
25 cc. of extract A		
grams	*cc.*	
37.7	1.00	Positive
35.2	0.70	Γositive
38.3	0.50	Positive
39.5	0.30	Positive
42.0	0.20	Positive
20 cc. extract B		
35.0	1.20	Positive
26.5	0.70	Positive
30.5	0.50	Doubtful
26.0	0.30	Negative
26.0	0.20	Negative
5 cc. extract C		
39.0	1.00	Positive
47.0	0.70	Positive
50.9	0.50	Positive
43.0	0.30	Positive
49.3	0.20	Positive

Since 0.2 extract A caused hyperexcitability (barely perceptible) in a frog
weighing 42.0 grams, 25 cc. of the extract would suffice to cause hyperexcitability
in frogs weighing in all 5.25 kgm., corresponding to 0.79 mgm. of strychnin
sulphate.

Since a dose of 0.7 cc. of extract B caused hyperexcitability in a frog weighing
26.5 grams, while 0.5 cc. was of doubtful effect when injected into a frog weighing
30.5 grams, we accept the latter dose as the minimal effective; this corresponds
to a total of 0.18 mgm. in 20 cc. of extract B (modified Stas-Otto).

A dose of 0.2 cc. of extract C caused hyperexcitability in a frog weighing
49.3 grams; 5 cc. would cause hyperexcitability in frogs weighing a total of
1.25 kgm., corresponding to a total of 0.19 mgm. of strychnin sulphate.

The greater amount of strychnin found in the total residues in this experiment indicates that the filter papers and the precipitates are all concerned in the loss during extraction. We wish to call special attention to the fact that filtration was avoided in all of the experiments of this series of studies except in those where it is specifically mentioned.

The loss due to filtration alone, is a variable factor in the extraction of alkaloids by the Stas-Otto method, and while we have not undertaken an extensive study of this phase of the problem, we have estimated the amount of strychnin taken up by the filter paper during the filtration of aqueous solutions of widely varying concentration. Four solutions of strychnin sulphate were filtered through Schleicher and Schuell soft filter papers no. 589 each weighing approximately 350 mgm., and measuring 7 cm. in diameter.

TABLE 5

Table showing the adsorption by filter paper of strychnin sulphate in aqueous solution

VOLUME OF SOLUTION	CONCENTRATION	LOSS OF STRYCHNIN	
cc.		mgm.	per cent
10	1:10,000	0.375	37.5
100	1:10,000	1.20	12.0
5	1:200,000	0.01	40.0
100	1:2,000,000	0.04	80.0

The tabulated results show that with a given concentration the loss increases with the volume, but the percentage loss decreases.

In contrast to the considerable loss that occurs during the filtration of aqueous solutions of strychnin sulphate, is the negligible loss during the filtration of a chloroformic solution of alkaloidal strychnin, as shown by the results of the following experiment.

A solution of 12.8 mgm. of strychnin sulphate, corresponding to 10 mgm. of the base, was rendered slightly alkaline and this

was shaken with 100 cc. of chloroform in successive portions. Ten cubic centimeter of the chloroformic solution, containing 1 mgm. of alkaloidal strychnin were diluted to 250 cc. with chloroform and this was filtered through ten Schleicher and Schuell filters no. 589 of 7 cm. diameter, which were folded once and placed one within the other so that the chloroform passed through all of them in succession. About 25 cc. of pure chloroform were then passed through the filters and this was mixed with the filtrate; the chloroform was distilled, and the residue was taken up in 50 cc. of normal salt solution containing a trace of sulphuric acid. The amount of strychnin sulphate present was estimated by means of the test on frogs; these tests indicated the recovery of all of the strychnin which was dissolved in the chloroform.

It is evident that the loss is negligible when a chloroformic solution of alkaloidal strychnin of moderate degree of concentration is filtered through a small filter paper such as was used in this experiment.

The gastric contents vary so widely at different times that a single experiment does not permit of drawing conclusions as to the relative merits of the two methods of extraction, but we have performed an experiment with a view to showing that very small amounts of strychnin can be recovered after admixture with the gastric contents of a cat.

Milk and meat were fed to a cat of medium size and after an interval of three hours the animal was killed, and the gastric contents, weighing 55 grams, were divided into two equal portions, A and B. One milligram of strychnin sulphate in 10 cc. of normal salt solution was mixed with each portion; after an interval of an hour these were extracted, A with sodium hydrate and immediate extraction with chloroform, B with acidulated alcohol in the manner already described with filtration where necessary.

The residue of A left after the distillation of the chloroform weighed 7 mgm. and the test on six frogs indicated that it contained more than 0.66 mgm. of strychnin sulphate. The resi-

due of B weighed 5 mgm., and the test on six frogs indicated that it contained less than 0.48 mgm.

The recovery of a larger percentage of the strychnin in this case by means of acidulated alcohol than in those experiments where the strychnin was added to muscle or blood is explained by the fact that there was much less solid matter present in the gastric contents.

Summary

1. The Stas-Otto method for the extraction of poisons from animal tissues does not permit of the recovery of strychnin quantitatively when only very small amounts (but such as may be present exceptionally at the time of death) are present, but widely diffused in the organs.

2. When one part of strychnin is present in ten million parts of tissue it may be extracted almost quantitatively by liquefying the tissue by means of sodium hydrate and heat, and shaking the liquid with chloroform. When the blood of an adult human being contains as much as half a milligram of strychnin at the time of death, the poison probably can be detected by the means which we have described.

3. When the residue left after the distillation of the chloroform is sufficient in amount to interfere with the absorbability of the poison from the lymph sac of the frog (or with the characteristic color reaction) this residue should be heated with concentrated sulphuric acid in order to destroy the organic matter other than strychnin, as suggested by Rodgers and Girdwood, after which the residue is rendered alkaline and extracted by chloroform.

4. Troublesome emulsions are sometimes formed, but the chloroform may be separated from such emulsions by shaking with chloroform or with water as circumstances require.

5. Strychnin is lost during the process of extraction from tissues through adsorption by insoluble residues and by filter paper when in acid solution in water and in alcohol, but chloroformic solutions of the base may be filtered through small papers virtually without loss.

III. ADSORPTION BY THE RED BLOOD CORPUSCLES IN THE CAT

There has been much discussion in the literature concerning the destruction of strychnin in the body, and while Hatcher and Eggleston (11) found that the alkaloid is destroyed at least in part in the liver they also suggested the possibility of the destruction of a part in the blood, but in one of their experiments they found that the strychnin which they had added to the blood of a dog and which had then been allowed to stand at body temperature during a period of forty-eight hours had not undergone destruction. They did not pursue the question of the destruction of the poison by the blood further.

We have presented evidence in the previous paper to show that strychnin can be recovered when extremely small amounts have been added to blood and we must assume, therefore, that the whole blood of the cat is incapable of destroying notable amounts of strychnin, but we believe that the results which we have obtained in the present investigation explain why Hatcher and Eggleston could not always recover the full amount of strychnin by the means that they employed after adding it to blood.

The results of our experiments designed to explain why acidulated alcohol fails to extract small amounts of strychnin from tissues quantitatively suggested that the red blood corpuscles play an important rôle in the disappearance of poisons from the circulation. They present extensive surface areas for adsorption, and the experiments described in this paper were intended to show whether such adsorption of strychnin occurs, if so, to what degree and whether strychnin so adsorbed is capable of exerting its typical action quantitatively when it is injected intravenously into the cat. We also hope to pursue the study of the influence of the red blood corpuscles on the elimination of strychnin and other alkaloids so soon as an opportunity presents itself.

Blood was withdrawn from the carotid artery and collected in a porcelain dish containing a known amount of strychnin dissolved in normal salt solution. Coagulation was prevented by the addition of potassium oxalate in the first of these experiments, by sodium citrate in the later ones.

Percentage of strychnin adsorbed

The specimens of diluted blood were allowed to stand for various periods of time after which the corpuscles were separated from the supernatant fluid in a centrifuge. The amounts of strychnin present in each were estimated separately by means of the biologic test on frogs after extraction in the manner already described in which the specimens were treated with an excess of sodium hydrate and shaken with chloroform.

Several protocols are given in brief to show the distribution of the strychnin after the addition of different amounts.

A large male cat was exsanguinated and 107.5 cc. of blood were added to an equal volume of normal salt solution containing 0.2 per cent of potassium oxalate. Half a milligram of strychnin sulphate in 0.5 cc. of normal salt solution was added, the mixture was shaken and allowed to stand half an hour. The diluted blood contained 1 part of strychnin sulphate in about 430,000 parts of the liquid. The separated corpuscles measured 52 cc., the supernatant fluid measured 163 cc.

The test of the chloroformic extract of the supernatant fluid indicated that it contained 0.37 mgm., that of the corpuscles, 0.12 mgm. of strychnin sulphate. Since the volume of supernatant fluid was about three times that of the corpuscles, it appears that the strychnin was distributed in these fractions in proportion to their respective volumes, but since the corpuscles contained relatively less fluid in proportion to the total volume, we must assume that at least a part of the strychnin present was adsorbed.

Eighty-four cubic centimeters of blood were withdrawn from a cat and mixed with an equal volume of normal salt solution containing 0.3 per cent of potassium oxalate. One milligram of strychnin sulphate, in 1 cc. of normal salt solution or 1 part of the poison to 169,000 parts of liquid, was added and the mixture was allowed to stand during half an hour, after which the corpuscles, measuring 58 cc., were separated from the supernatant fluid which measured 110 cc.

The tests on frogs indicated that the corpuscles held about 0.34 mgm. of strychnin sulphate, while the supernatant fluid

held about 0.46 mgm.   The results of this experiment indicate
that with an increase in the concentration of strychnin in the
diluted blood the corpucles adsorbed a larger percentage.   The
supernatant fluid held 1 part in about 240,000 parts, while the
corpuscles held 1 part in about 170,000 parts.

In the succeeding experiment 2 mgm. of strychnin were added
to a mixture consisting of 62 cc. of oxalated blood and 62 cc. of
normal salt solution, the concentration being equal to 1 part in
about 62,000 parts of liquid.   The mixture stood half an hour,
after which the corpuscles, measuring 31 cc., were separated
from 93 cc. of supernatant fluid.   The tests indicated that the
corpuscles held 1.28 mgm. (corrected to 1.2 mgm.) of strychnin
sulphate, a concentration of 1 part to about 25,000 parts, and
that the supernatant fluid held 0.92 mgm., a concentration of
1 part in about 100,000 parts.

Three milligrams of strychnin sulphate were added to a mix-
ture consisting of 68 cc. of blood to which 0.68 gram sodium
citrate had been added, and 68 cc. normal salt solution,[3] the con-
centration of strychnin being equal to 1 part in 45,333.   After
an interval of ten minutes the corpuscles, measuring 31 cc., were
separated from 105 cc. of supernatant fluid.   The tests indicated
that the supernatant fluid contained 1.35 mgm. of the strych-
nin, the concentration being 1 part in about 77,700, while the
corpuscles retained 1.46 mgm., the concentration being equal
to 1 part in about 21,300.   It may be only accidental that with
an increase in the concentration of strychnin in the diluted
blood, the corpuscles appear to have retained relatively less
than in the previous experiment in which, however, the mix-
ture was allowed to stand thirty minutes before being centri-
fuged.   At any rate, the duration of this period beyond a mini-
mum of ten minutes does not appear to influence the distribution
of the strychnin between the corpuscles and the supernatant

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[3] The strychnin sulphate was dissolved in normal salt solution and this was
added to the blood in nearly all of these experiments; and while we speak of
"citrated blood" for convenience, the sodium citrate was usually dissolved in
the normal salt solution and this was added to the blood at once.   The normal
salt solution in which the strychnin was dissolved is included in the amount
stated as having been added to the blood.

fluid to any marked degree, and it is almost certain that there are other factors that exert a greater influence on this distribution than does the further increase in the period of contact before separating the corpuscle and the supernatant fluid. The results show a striking similarity in distribution in the next two experiments in which the concentration was nearly the same, while the period of contact was widely different.

In the first of these two experiments 4 mgm. of strychnin sulphate were added to a mixture consisting of 70 cc. of citrated blood and 70 cc. of normal salt solution, the concentration of the strychnin being equal to 1 part in 35,000 parts of fluid. After an interval of ten minutes the corpuscles, measuring 40 cc., were separated from the supernatant fluid, which measured 100 cc. The tests on frogs indicated that the supernatant fluid contained 1.68 mgm., the concentration being equal to 1 part in about 60,000 parts of fluid, while the corpuscles held 2 mgm., the concentration being 1 part in 20,000 parts.

In the second of these two experiments 4 mgm. of strychnin sulphate were added to a mixture consisting of 72 cc. of citrated blood and 76 cc. of normal salt solution, the concentration being equal to 1 part in about 37,000 parts of fluid. After a period of three hours, during which the mixture was shaken occasionally, the corpuscles were separated from the supernatant fluid. The distribution of strychnin between the corpuscles and the supernatant fluid was almost exactly the same as in the previous experiment. The tests indicated that the corpuscles retained 2.1 mgm., that the supernatant fluid held 1.52 mgm.

Retention of strychnin by washed corpuscles

Two experiments were performed for the purpose of learning whether strychnin which has become fixed by the red blood corpuscles of the cat can be removed readily by washing them with citrated normal salt solution.

In one of these two experiments 3 mgm. of strychnin sulphate were added to 69 cc. of citrated blood with 71 cc. of normal salt solution; after an interval of ten minutes 500 cc. of citrated normal salt solution were added, the mixture was allowed to

stand during ten minutes, after which the corpuscles were sep-
arated; they were subsequently washed twice, 500 cc. of citrated
normal salt solution being used for each washing. The cor-
puscles were extracted and the amount of strychnin retained
by them was estimated in the usual way. The tests indicated
that they retained about 1.5 mgm., or about 50 per cent of the
strychnin that had been added to the blood.

Four milligrams of strychnin sulphate were added to 72 cc.
of citrated blood and 80 cc. of normal solution in the second
of these experiments; the mixture was allowed to stand during
ten minutes, after which 1000 cc. of citrated normal salt solu-
tion were added and the mixture was allowed to stand with
occasional stirring during six hours; the supernatant liquid was
decanted, and 500 cc. of citrated normal salt solution were added
to the corpuscles; after ten minutes the corpuscles were separated
in the centrifuge, and they, and the mixed plasma and washings,
were extracted separately. The tests indicated that the cor-
puscles retained 1.63 mgm. to 2 mgm. of the strychnin sulphate,
that the plasma and washings contained 2 mgm.

The results of these two experiments show that when 1 part of
strychnin sulphate is added to about 20,000 parts of blood of
the cat the corpuscles fix about 50 per cent of it in such a
way that it cannot be separated readily by washing them with
citrated normal salt solution.

Rôle of the stroma

The question presented itself to us whether the strychnin which
is fixed by the red blood corpuscles is attached to the stroma of
the cells or penetrates into the cells to be held in solution or
attached to the hemoglobin. Three experiments were performed
the results of which throw some light on the problem, but we
have not learned whether the hemoglobin plays any part in the
fixation of this strychnin.

Three cats were exsanguinated and 2.1 grams of sodium citrate
were added to 210 cc. of the mixed blood thus obtained, which
was then divided into three equal portions, A, B, and C. These
three portions were treated alike except for the fact that 2 mgm.

of strychnin sulphate were added to A, and the same amount to B, while three milligrams were added to C. The procedure was in every case as follows:

The blood was allowed to stand during fifteen minutes after the addition of the strychnin; the corpuscles were separated in

TABLE 6

Showing the amounts of strychnin fixed by the red blood corpuscles after addition to diluted blood

EXPERIMENT NUMBER	VOLUME OF DILUTED BLOOD	VOLUME OF CORPUSCLES	AMOUNT OF STRYCHNIN ADDED	AMOUNT OF STRYCHNIN FOUND IN CORPUSCLES	
	cc.	cc.	mgm.	mgm.	per cent
1	215	52	0.5	0.12	24
2	169	58	1.0	0.34	34
3	124	31	2.0	1.2	60
4	136	31	3.0	1.46	49
5	140	40	4.0	2.0	50
6	148		4.0	2.1	52
Corpuscles washed with citrated normal salt solution					
7	140		3.0	1.5	50
8	152		4.0	1.8	45

TABLE 7

Showing the amounts of strychnin fixed by the red blood corpuscles of the cat, and the percentage that is retained by the stroma

	AMOUNT OF STRYCHNIN ADDED TO BLOOD	AMOUNT FOUND IN CORPUSCLES		AMOUNTS OF STRYCHNIN FOUND	
				In stroma	Dissolved
	mgm.	per cent	mgm.	mgm.	mgm.
A	2.0	47.5	0.95	0.57	0.38
B	2.0	46.0	0.92	0.55	0.37
C	3.0	48.0	1.44	1.22	0.22

the centrifuge and were then washed with 50 cc. of citrated normal salt solution, after which they were again separated in the centrifuge and dissolved by the addition of 200 cc. of distilled water. After an interval of thirty minutes the insoluble residue, consisting of the stroma of the corpuscles, was separated in the centrifuge, the residue measuring about 5 cc., the super-

natant liquid about 225 cc. The latter was evaporated on a water bath, after which this evaporated portion and the insoluble residue (stroma) were extracted separately and the amounts of strychnin present in each were estimated in the usual way.

The tabulated results show the amounts of strychnin found in the stroma and in the evaporated liquid of each of the several portions, expressed in fractions of a milligram; and the percentages of the amounts that were added to the blood which were found in the corpuscles.[4]

It is evident that the stroma retain the greater part of the strychnin which is fixed by the red blood corpuscles, but we have no satisfactory explanation to offer that will explain the much higher percentage found in the experiment C. The lower content found in the liquid portion in this experiment is in harmony with the result obtained with the stroma, however, we are inclined to believe that the result is due to a more perfect separation of the stroma. In every one of these three experiments the total amount of strychnin found in the corpuscles (stroma and solution) is equal to about 50 per cent of that added to the blood, these results agreeing closely with those obtained in previous experiments.

Relative toxicity of poisoned plasma and corpuscles for cats

The tenacity with which strychnin is held by the red blood corpuscles suggests that this factor may play an important rôle in the development of the action of strychnin, and we undertook to determine whether that which is fixed by the red blood corpuscles is capable of inducing its typical action quantitatively when it is injected intravenously into another cat. Two experiments were performed with this object in view, but we will present the protocol of only one of them in brief, since the results in the other experiment were closely similar.

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[4] We have not overlooked the fact that the personal element is concerned in the determinations of the minimal effective dose, and we have endeavored to minimize this factor in the way explained in the first paper of this series. We could not know what amounts of strychnin were indicated at the time that we determined the minimal fatal doses in the present experiments, and we were somewhat surprised later to find how close the agreement is.

Seventeen milligrams of strychnin sulphate dissolved in 17 cc. of normal salt solution were added to 170 cc. of mixed blood obtained from two cats, and 1.7 grams of sodium citrate were dissolved in the mixture which was allowed to stand during one hour, after which it was centrifuged, the separated corpuscles measuring 55 cc., and the supernatant liquid 132 cc.

Numerous experiments had shown that the corpuscles retained about half of the strychnin that had been added to the blood in amounts such as were used in this experiment, but a specimen of the corpuscles was extracted and the amount present was estimated in the usual way. The tests indicated that the corpuscles retained 7.4 mgm. of strychnin sulphate. The remainder of the corpuscles was diluted with an equal volume of normal salt solution, and this, and the supernatant liquid were tested separately by intravenous injection into cats.

An average of 2.65 cc. of the supernatant liquid per kilogram of weight was required to cause death in four experiments, indicating that the total, 132 cc., would suffice to cause death in cats weighing in the aggregate 60 kgm. This corresponds to a total of 9.7 mgm. of strychnin sulphate, if the activity in this solution is the same as that in normal salt solution when injected at the same rate, as shown in two experiments. An average of 5 cc. of the diluted corpuscles per kilogram of weight was required to cause death in four experiments, indicating that the total, 110 cc., of diluted corpuscles, containing 7.4 mgm. of strychnin sulphate, would suffice to kill cats weighing in the aggregate 22 kgm.

The fatal intravenous dose of strychnin sulphate was 0.19 mgm. per kilogram of weight when the supernatant liquid was used, and 0.33 mgm. when the diluted corpuscles were used, showing that the corpuscles do not give up the strychnin to the tissues as readily as the serum of the blood.

The results in the second of these two experiments are capable of being so interpreted as to indicate an even greater difference between the activity of the strychnin present in the corpuscles and that of the supernatant liquid but the true ratio appears to

be almost exactly similar to that shown in the experiment just detailed.

It is well known that the activity of strychnin depends to some degree on the rate at which it enters the circulation and on the concentration of the solutions employed, we were careful, therefore, to inject the diluted corpuscles and the supernatant liquid at corresponding rates in these experiments.

In two of the experiments nearly all of the calculated dose of the poisoned corpuscles was injected within the first five minutes, after which the injection was made at a slower rate. In the other two experiments with diluted corpuscles the initial rate of injection was one third of that in the first two, so that more than fifteen minutes were required for the injection of the calculated fatal dose. The supernatant liquid was injected at corresponding rates, in two experiments the injection being comparatively rapid, in two, one third as fast. The average duration of the injection of the diluted corpuscles was 38 minutse that of the supernatant liquid was twenty-three minutes.

We have not studied the rôle of the red blood corpuscles in the elimination of poisons from the animal body, except insofar as they may be concerned in the disappearance of strychnin from the circulating blood, but it seems probable that they are concerned with the distribution of strychnin in the body after it enters the blood, and it is obvious that they do give up at least a part of their attached strychnin readily to those structures upon which its toxic action is exerted, and including the spinal cord, the respiratory center and the vaso-motor centers, while they resist its removal during washing with normal salt solution.

It would be interesting to know whether the cat is more resistant toward strychnin which is fixed by the red blood corpuscles than it is toward that which is dissolved in the plasma because the structures just mentioned take it up less readily than they do that which is dissolved in the plasma, or because the liver takes it more readily. We hope, as previously stated, to study this question in the near future.

Summary

1. When 1 part of strychnin sulphate is added to 430,000 parts of a mixture of oxalated or citrated blood and an equal volume of normal salt solution, the strychnin is distributed between the corpuscles and the plasma nearly in proportion to their respective volumes.

2. With increasing concentrations up to that of 1 part of the poison in 60,000 parts of diluted blood, the corpuscles fix increasing percentages of the strychnin, so that when this concentration is reached the corpuscles fix about three times as much as the plasma holds in proportion to their volume.

3. When strychnin is added to blood in the higher concentration fixation by the corpuscles appears to be maximum within ten minutes, at least, there is no apparent difference between the amounts fixed in ten minutes and those fixed in one hour.

4. The red blood corpuscles fix approximately one-half of the strychnin sulphate which is added in the proportion of 1 part to 20,000 parts of citrated blood and diluted with an equal volume of salt solution.

5. Strychnin which has become fixed by the red blood corpuscles in this way is not removed when the corpuscles are washed repeatedly with citrated normal salt solution.

6. When distilled water is added to the red blood corpuscles, causing hemolysis, after they have fixed strychnin, the stroma retain the greater part of the poison.

7. Strychnin which has been fixed by the red corpuscles does not exert its typical action quantitatively so promptly after its intravenous injection into the cat as that which is held in solution in the plasma, and approximately 50 per cent more of such adsorbed strychnin is required to cause death promptly after its intravenous injection than of that which is held in the citrated plasma.

IV. THE RATE OF DISAPPEARANCE OF STRYCHNIN SULPHATE FROM THE BLOOD STREAM OF THE CAT

The importance of a knowledge of the rate of disappearance of poisons from the circulation is evident, but our knowledge of this subject is fragmentary and investigators have concerned them-

selves mainly with the problem of the ultimate fate of such substances after their introduction into the body, and while we have a considerable store of information regarding the destruction of various substances in the tissues, or their elimination in the excreta, much remains to be learned of the details of the excretion, or destruction in the body of many of the therepeutic agents in common use.

The lack of exact knowledge of the several steps by which active substances leave the circulation, exert their typical actions on various structures, and finally leave the organism, results in failure to use them to the greatest possible therapeutic advantage, and to treat patients most effectively when poisoning results from overdosage, however this has been brought about.

The concentration in which a drug exists in the circulation often influences its distribution in the tissues, and consequently the nature of its action, to a greater degree than does the total amount which is absorbed in a unit of time. Instances will occur to every pharmacologist, but it may be mentioned that Hatcher and Eggleston (15) found that while similar amounts of morphin sulphate are required to cause restlessness in the cat, whether the poison be administered intravenously, intramuscularly, subcutaneously, or by the stomach, the dose required to produce distinct analgesia after subcutaneous or intramuscular injection is more than thirteen times as large as that required to induce this effect after intravenous injection.

It is said that some drugs remain for long periods in the circulation, while other substances are said to leave the blood with a rapidity which is almost incredible. There is no question that different agents show wide differences in this respect, and that animals of different species get rid of poisons at widely different rates. It is impossible, however, in the present state of our knowledge to classify drugs satisfactorily with reference to their behavior, and every drug must be studied individually.

We have used the cat in the present investigation partly because of the convenience which its use affords, partly because we have come to consider the behavior of this animal toward numerous poisons as resembling the behavior of man toward

the same substances more closely than does that of any other animal commonly used for experimental purposes. We do not know that strychnin leaves the blood stream in man exactly as it does in the cat, but the resemblance between the general behavior of man and the cat toward strychnin leads us to believe that the results of our experiments on cats can be accepted as probably showing the approximate rate of disappearance of strychnin from the human circulation. All except two of the cats were full grown, the weights of the latter varying from 2.58 to 3.4 kgm.

The experiments were conducted in the following manner: Strychnin sulphate in solution was injected into the femoral vein in doses of 1 mgm. for each kilogram of body weight, and after varying intervals of time blood was drawn from the carotid artery so long as it would flow freely, or until a sufficient amount had been obtained. Sodium citrate was added to prevent coagulation, and in the greater number of the experiments the blood was diluted with normal salt solution, after which the corpuscles were separated from the plasma and the amounts of strychnin present in the plasma and corpuscles were determined in the manner described in a previous paper of this series. The blood was defibrinated in two of the experiments. The animals were kept alive by means of artificial respiration and the administration of chloroform through a tracheal cannula until the blood was drawn. The strychnin is probably held in the body in the form of a base, but it is more convenient to speak of this in terms of the sulphate.

The total amount of strychnin present in the blood after the lapse of the interval selected was estimated in the following way: The amount present in a measured specimen of the drawn blood was determined by means of the frog test in the manner previously described and from this the amount present in the total blood of the animal was calculated. It is now commonly accepted that the blood of the cat equals 5 per cent of the total body weight. The specific gravity of blood is approximately 1.050, hence the weight of the body in grams, multiplied by $(0.05 \div 1.050 =) 0.0476$ gives the estimated volume of the blood in cubic centimeters.

The proportion of the blood varies somewhat in different individuals, but this variation is not sufficient to interfere with the value of the determinations of the rate of elimination of poisons from the blood.

The distribution of the strychnin between the corpuscles and plasma was studied in the hope that it would throw some light on the problem of the disappearance of the poison from the circulation, its further distribution in the body, and its elimination. We hope to use the data thus obtained in studies which we plan to pursue later.

Experiments

Protocols of several experiments will be given in brief.

A cat weighing 2.58 kgm. received an intravenous injection of 2.6 mgm. of strychnin sulphate, and after an interval of two minutes (during which chloroform was administered and artificial respiration was maintained) 60 cc. of blood were withdrawn from the carotid artery; the blood was defibrinated; the corpuscles were separated in a centrifuge, after which they were washed with 50 cc. of normal salt solution; the fibrin was washed and the washings of the corpuscles and that of the fibrin were added to the serum.

The corpuscles, the fibrin, and the serum, together with the washings, were extracted; the chloroformic extract of the corpuscles was dissolved in 10 cc. of normal salt solution, that of the fibrin in 10 cc., and that of the serum and washings in 15 cc., a trace of sulphuric acid being used in every case to effect solution. These were tested on frogs in the manner previously described; the results of these tests indicate that the corpuscles retained about 0.36 mgm. of strychnin sulphate; the fibrin about 0.05 mgm., and the serum and washings about 0.79 mgm., or a total of 1.2 mgm. in the 60 cc. of drawn blood.

The total volume of the blood of the cat was estimated to be (2580×0.0476) 122.4 cc., and since the tests indicated that 60 cc. of drawn blood contained 1.2 mgm., this would indicate that the total blood contained 2.4 mgm., or practicaly all that

was injected. There are several possible explanations to account for this result, and it is probable that more than one of these is actually involved.

It seems probable that the intense vaso-constriction which this dose of strychnin induced interfered with the distribution of the strychnin, and that the blood that was drawn within two minutes after the injection contained a larger proportion of the poison than the blood which remained in the muscles. Obviously if the drawn blood did contain a greater proportion of the strychnin, then a greater amount had left the circulation than the results of the tests indicate. It is difficult to believe, however, that this is the chief factor in the explanation, and we are inclined to think that the indicated strychnin content of the serum is too high, owing to an unusual susceptibility of one or two of the frogs used in the test.

It is almost certain that more of the strychnin than is indicated by the tests had left the blood stream in this period, and the distribution of the poison between the corpuscles and plasma is so different from that which we found in vitro with an approximately similar concentration, that we are forced to the conclusion that the results in this experiment should be discarded. The results of the later experiments, as stated, are not open to these objections, and we must suppose that a considerable amount of strychnin leaves the blood stream within two minutes after its intravenous injection.

The experiment was repeated with a cat of the same weight, and a similar interval of time was allowed to elapse after the injection before the blood, measuring 48 cc., was withdrawn, but the coagulation of the blood was prevented by the addition of sodium citrate. The results of the tests on frogs indicated that the corpuscles of the 48 cc. of drawn blood retained more than 0.35 mgm., less than 0.58 mgm. of strychnin sulphate, while the plasma contained more than 0.20 mgm., less than 0.23 mgm., a total of more than 0.55 mgm., less than 0.81 mgm., corresponding to more than 1.4 mgm., less than 2.06 mgm. in the total blood. If we accept the mean of these two—1.73 mgm.—we must suppose that 32 per cent of the strychnin

had left the blood within a period of two minutes following its injection.

Two experiments were performed with intervals of five minutes following the injection before the blood was withdrawn. The results of the tests on frogs indicate that about 59 per cent of the strychnin that was injected had left the circulation in the first of these, and about 64 per cent in the second.

Two experiments similar to these were performed, but young cats were used for the purpose of comparing the rate of elimination of strychnin from the circulation in these with that in adults. The smaller of these weighed 1.54 kgm., the larger weighed 1.68 kgm., but both were obviously young, and not merely light in weight. The results of the tests of the extracts on frogs indicate that 85 per cent of the injected strychnin left the circulation of the smaller cat in five minutes, and that 82 per cent left the circulation of the larger of the two; no significance can be attached to the slight difference in percentage in the two experiments, but it is important to observe that in both of these the rate of disappearance from the circulation was distinctly greater than in the adult cats. This difference can hardy be a mere coincidence.

The more rapid disappearance in the young cats may be due to greater activity of the liver in fixing it, and it is well known that very young animals (much younger than those used by us) are more tolerant than adults of the same species toward strychnin.

Two experiments were performed in which intervals of ten minutes followed the injection before the blood was withdrawn. The results of the tests indicate that about 66 per cent of the poison had left the blood in the first of these, and that about 78 per cent in the other.

In one experiment, in which an interval of twenty minutes followed the injection of the strychnin before the blood was withdrawn, the tests indicated that 88 per cent of the poison had disappeared from the blood, and in one in which the interval was forty minutes, no evidences of the presence of strychnin in the plasma could be obtained, and the corpuscles retained only about four per cent of that which had been injected.

TABLE 8

Table showing the elimination of strychnin from the blood, the concentration in the blood, and the distribution between plasma and corpuscles after varying intervals of time following its intravenous injection

WEIGHT	INTERVAL	BLOOD DRAWN	STRYCHNIN IN BLOOD DRAWN	ESTIMATED TOTAL BLOOD IN BODY	STRYCHNIN IN BLOOD	STRYCHNIN INJECTED	STRYCHNIN ELIMINATED IN PERCENTAGE OF THAT INJECTED	PARTS OF BLOOD BY VOLUME TO ONE PART OF STRYCHNIN BY WEIGHT, AFTER EXPIRATION OF INTERVAL	PERCENTAGE OF STRYCHNIN FOUND: IN CORPUSCLES	PERCENTAGE OF STRYCHNIN FOUND: IN PLASMA
kgm.	minutes	cc.	mgm.	cc.	mgm.	mgm.				
2.58	2	60	1.20	122.4	2.45	2.6	6	50,000	30	70*
2.58	2	48	0.68	122.4	1.73	2.6	33	70,000	68	32
3.10	5	50	0.43	147.5	1.27	3.1	59	116,000	65	35
3.40	5	60	0.45	161.8	1.22	3.4	64	133,000	52	48
3.00	10	49	0.35	142.8	1.02	3.0	66	140,000	46	54*
2.80	10	69	0.32	133.3	0.62	2.8	78	216,000	34	66
2.84	20	48	0.13	135.2	0.35	2.8	88	370,000	79	21
2.80	40	50	0.04	133.3	0.11	2.8	96	1,250,000	97	−3
Young cats										
1.54	5	34	0.10	73.3	0.22	1.5	85	340,000	35	65
1.68	5	34	0.12	80.0	0.28	1.6	82	283,000	30	70

* The drawn blood was defibrinated in this experiment.

Discussion

These results hardly require detailed discussion, but one may say that when strychnin sulphate enters the circulation it is at first held in solution in the plasma from which part of it passes rapidly into the corpuscles, part of it into the tissues. When the concentration in the plasma is reduced to a certain undetermined point, which probably varies in different individuals, the poison begins to pass from the corpuscles back into the plasma.

We stated in the third paper of this series that the percentage of the strychnin fixed by the corpuscles in vitro increases with the concentration in the whole blood up to a certain point, and that the strychnin so fixed is not removed readily when the corpuscles are washed with citrated normal salt solution.

The results in the last experiment of this series agree, with those obtained in vitro, for the corpuscles retained a notable amount of the poison after the lapse of forty minutes though the plasma contained less than 0.004 mgm., corresponding to a dilution of 1 part in approximately in 12,000,000 but the tissues concerned with its elimination or destruction were able to take it readily from the plasma even in that dilution.   In view of the rapidity with which the greater part of the strychnin leaves the circulation we must suppose that the organs concerned with its elimination must remove during each circuit of the blood practically all of the poison from the plasma of that part of the blood which comes to them, and that the strychnin would disappear from the blood even more rapidly than it does were it not fixed in the corpuscles promptly and held firmly.

The distribution of the strychnin between the corpuscles and plasma of the young cats is of interest, since it appears that the corpuscles held relatively less of the strychnin than in the case of the adults with approximately similar concentrations, but we are unable to say whether this is the cause or the effect of the more rapid disappearance of the strychnin which certainly took place in these young animals.   It will be interesting to learn the comparative readiness with which the poisoned plasma and corpuscles give up strychnin to the liver during perfusion, and the readiness with which each of these removes it when that organ is perfused with unpoisoned plasma or suspension of corpuscles.

We do not claim that we have determined the amounts of strychnin present in the blood of the cats with a degree of precision commensurate with the delicacy of the test in detecting small amounts of strychnin in tissues, and it is probable that the error averages about 10 per cent, but the constant diminution in the amounts found in the blood with increasing intervals of time following the injection of the poison, indicates that any such error in these estimations does not lessen the value of the results.   We wish to direct attention again to the results obtained in the last experiment because of their evident importance in the problem of the elimination of strychnin from the

body, and to repeat that while the corpuscles contained a notable amount of the poison—nearly four per cent of that injected—it is quite certain that the plasma did not contain more than traces, probably less than 1 part in 12,000,000.

Summary

1. Strychnin was injected intravenously into cats in doses of 1 mgm. per kilogram of weight, and after intervals of time, varying from two minutes to forty minutes, blood was withdrawn from the carotid artery in measured amounts, and the percentages of strychnin present in the corpuscles and plasma (or serum) were determined separately in the manner described in a previous paper.

2. Strychnin sulphate leaves the blood stream rapidly, and after 2 minutes as much as 30 per cent may have left the circulation; within five minutes more than 50 per cent, and after forty minutes the blood may contain only about 4 per cent of that injected.

3. Young animals appear to eliminate strychnin from the circulation more rapidly than adults; this probably stands in relation with the greater tolerance of young animals toward strychnin, but the difference in the rate of elimination found in these experiments is greater than the difference in tolerance of animals of the age used and that of adults.

4. When strychnin sulphate is injected intravenously into the cat the poison passes from the plasma into the tissues in part, in part into the corpuscles, and after a time it begins to pass from the corpuscles back into the plasma and thence into the tissues, and in one experiment after an interval of forty minutes the corpuscles were found to contain about 4 per cent of the strychnin injected, while the plasma contained only traces, the concentration in the plasma being equal to 1 part in about 12,000,000.

5. The distribution of strychnin between the plasma (or serum) and the corpuscles following its intravenous injection into the cat does not appear to differ materially from that seen when the poison is added to the blood in vitro in an approximately similar concentration.

6. It is believed that the determination of the distribution of the poison between the plasma and the corpuscles will aid in the study of the behavior of strychnin in the body, and expecially of its elimination, by means of perfusion experiments.

V. RATE OF ELIMINATION OF STRYCHNIN SULPHATE IN THE URINE OF MAN

It was formerly held that strychnin is eliminated by the kidneys almost quantitatively, but Hatcher and Eggleston (11) called attention to the fact that this view is not supported by any experimental evidence of which they could learn, and they found that only a small fraction of the strychnin which they administered to cats, dogs and guinea-pigs could be recovered in the urine and in the feces, the larger part being destroyed in the body; they also found that it is destroyed in the liver during perfusion with diluted defibrinated blood to which the poison has been added. They were unable to detect the poison in the urine of a guinea pig, after twenty-four hours following the administration of a total of 21 mgm. in a period of twenty-four hours, and their experiments show that cats and dogs recover completely within a few hours after the administration of toxic doses, even when such doses are given several times daily for periods up to two weeks.

Strychnin has been detected in the urine within a few minutes after its administration in numerous instances, and von Rautenfeld (1) stated that he found it in the urines of two very old patients six days after completing the administration of a total of 5 mgm. of the nitrate to each.

It is evident that animals of different species differ greatly in their capacities for eliminating strychnin, and consideration of the observations of von Rautenfeld and others suggests that man shows considerable variation in capacity for excreting and decomposing this poison; we have therefore recorded the available data that may serve to throw some light on the conditions that influence the rate of elimination of strychnin, and on the percentage of the total dose which is eliminated by the kidneys in man, and it is to be observed that a high initial rate of elim-

ination does not necessarily mean a correspondingly high total percentage that ultimately appears in the urine.

The authors served as the subjects of these experiments because of the difficulty of insuring accuracy of dosage and time of collection of urine when these are intrusted to others who do not appreciate the necessity of precision in performing duties that are commonly a matter of routine and where slight variations are without significance.

The experiments were performed during the spring, at which time both H. and W. were actively engaged in laboratory work, the duties of W. taking him out of doors somewhat more than those of H. did him.

H. was fifty-three years of age, in good health, but decidedly under the average weight, his height being 1.73 meters and weight about 61 kgm. He had had measles, varicella, pertussis, and pneumonia previous to the age of twelve years, an appendectomy with extensive infection, at thirty-one, typhoid fever at thirty-six years, parotitis at forty-six, and no serious illness since that time. The diet is mainly vegetable, meat is taken in moderation two or three times a week; tea, cocoa and milk are used freely, but no other liquids are taken habitually (pure water being taken only infrequently).

W. was twenty-one years of age, in good health, but much under the average weight, his height being 1.87 meters and his weight 70 kgm. He had had measles and varicella before the age of ten years, since which time he has had no serious illness. Meat is eaten in moderation daily; coffee is taken at every meal; no other liquid except water is used.

The bladder was emptied completely at the moment when the first dose of strychnin of the experiment was taken, and the urine was then collected in clean bottles for periods of six, twelve, or twenty-four hours, according to the requirements of the experiment. Therapeutic doses were used. These were administered orally in single and repeated doses, and intramuscularly in single doses. Nearly similar experiments were employed with each subject.

The extraction of the strychnin was performed in the following manner: The urine, rendered strongly alkaline by the addition of KOH, was evaporated in a porcelain dish to about one tenth of the original volume, the residue was shaken several times with somewhat larger volumes of chloroform which were mixed and distilled; the residue was dissolved in water to which a little sulphuric acid had been added, this was rendered alkaline, and the mixture was shaken three times with somewhat larger volumes of chloroform as before; the mixed chloroformic extracts were distilled, and the residue was taken up in a few cubic centimeters of normal salt solution to which a trace of sulphuric acid had been added. The amount of strychnin present was determined by means of the frog test in the manner already described.

We accepted the mean of the lowest effective dose used and the highest ineffective, in the tests on frogs, as being the true minimum required to produce hyperexcitability, and this is used in calculating the amount of strychnin present in any specimen of urine, except in those cases where the degree of hyperexcitability was so slight after the smallest effective dose, as to indicate that it was very near the minimum that would be effective; in such cases it was accepted as the minimum.

Several preliminary experiments were performed in order to determine the availability of the method for our investigation. In these strychnin sulphate was added to half of the urine and none to the other half; the resulting extracts were injected into frogs which were then observed by one of us who did not know what had been injected. In two of these control experiments, in each of which 0.01 mgm. of strychnin had been added to 1200 cc. of urine, the extract caused typically increased reflexes, while the extract of the unpoisoned urine produced no perceptible effect. Similar results were obtained in all of these preliminary experiments.

One can say that strychnin can be detected with certainty when less than 0.01 mgm. is present in a twenty-four hour specimen of urine of a normal adult. We have not experimented with urines obtained from those suffering from disease, but it

seems altogether probable to us that the method is equally applicable in pathologic conditions.

We are more sharply limited by the minimum amounts of strychnin that can be detected with certainty than we are by the dilution in which slightly larger amounts exist. The smallest frogs usually available weigh about 10 grams, and approximately 0.002 mgm. of strychnin sulphate is required to induce increased reflexes in a frog of that weight, and the difficulty of purifying very small amounts solutions hardly calls for discussion.

Strychnin is extremely resistant to decomposition by the reagents and by the operative procedure usually employed for extraction, hence one can recover strychnin and detect it with certainty, when moderate amounts—1 mgm., or more—are present in enormous amounts of urine and the dilution in water in which it can be detected appears to be limited only by the time which one can devote to the evaporation of the solution.

Experiments

The first four experiments were carried out in a nearly similar manner, except that active diuresis was induced in the third and fourth. In all of these a singe dose of 4 mgm. dissolved in 1 cc. of water was injected into the deltoid muscle. In the sixth and seventh experiments single oral doses of 4 mgm. with 36 mgm. of lactose, each in a gelatin capsule, were taken in the morning on rising. In the seventh a total of 13 mgm. of strychnin sulphate was taken in the form of tablets of $\frac{1}{30}$ grain (2.2 mgm.), one being administered at each dose; these were taken at 8.00 and 11.00 a.m., and at 2.00, 5.00, 8.00, and 11.00 p.m. In the last experiment a total of 15 mgm. of strychnin sulphate in similar tablets was taken, one tablet being administered at 8.30 and 11.30 a.m., at 2.30 and 5.30 p.m., and at 6.30 and 9.30 a.m., and 12.30 p.m. of the following day, respectively, the doses being thus spaced in this experiment because of a tendency toward headache.

Diuresis was induced in two experiments by copious drinking of tea, cocoa, lemonade and hot water by H. and of lemonade by W.

The protocol in brief of one experiment will be given and the results of all of the experiments will be tabulated.

Protocol of experiment 3 (in brief).   Intramuscular injection—diuresis. Cocoa and water taken abundantly during the first six hours.

11.10 a.m.   4.0 mgm. strychnin sulphate in 1 cc. normal salt solution
              injected into deltoid of W.

 5.10 p.m. 2200 cc. urine collected, specimen A 6 hours

11.10 p.m.   210 cc. urine collected, specimen B 6 hours

11.10 a.m.   450 cc. urine collected, specimen C 12 hours

11.10 a.m. 1020 cc. urine collected, specimen D 24 hours

11.10 a.m. 1032 cc. urine collected, specimen E 24 hours

The several specimens of urine were extracted (in the manner described) and the extracts dissolved in normal salt solution containing a trace of sulphuric acid: A in 20 cc.; B in 5 cc.; C in 3 cc.; D in 2.5 cc.; and E in 1 cc; the tests on frogs follow:

SPECIMEN	WEIGHT OF FROG	DOSE	RESULT
	grams	*cc.*	
A. 20.0 cc.	56.5	0.50	Positive
	40.1	0.30	Borderline
	34.2	0.2	Negative
	35.3	0.15	Negative
B. 5.0 cc.	33.5	0.80	Positive
	33.4	0.50	Negative
	33.1	0.30	Negative
	29.2	0.20	Negative
	25.1	0.10	Negative
C. 3.0 cc.	19.5	1.0	Positive
	14.3	0.50	Positive
	18.5	0.30	Positive
	18.5	0.15	Negative
D. 2.5 cc.	13.2	1.0	Positive
	13.8	0.50	Positive
	10.5	0.30	Positive
	12.8	0.15	Minimum
E. 1.0 cc.	40.5	1.0	Negative

Calculations (0.15 mgm. strychnin sulphate per kilogram of frog causes increased reflexes):

A.   A dose of 0.3 cc. caused minimal increased reflexes in A frog weighing 40.1 grams; 20 cc. would cause increased reflexes in frogs

TABLE 9

Table showing the rate of elimination of strychnin sulphate by the kidneys in successive intervals of time following the intramuscular and oral administration of therapeutic doses in man

SUBJECT	DOSE	INTERVAL	AMOUNT OF STRYCHNIN SULPHATE FOUND:		VOLUME OF URINE
			In fractions of a milligram	In percentage of dose	
After intramuscular injection					
	mgm.	*hours*			*cc.*
W	4	6	0.3S	9.5	320
		6	0.20	5.0	325
		12	0.075	1.9	350
		24	0.005	0.1	1050
		48	0.66	16.5	2045
H	4	6	0.25	6.2	550
		1S	0.51	12.S	S00
		24	0.005	0.1	1053
		24	(−0.006)		1070
		72	0.765	19.1	3473
Diuresis after intramuscular injection					
W	4	6	0.40	10.0	2200
		6	0 04	1.0	210
		12	0.04	1.0	450
		24	0.03	0.S	1020
		24	(−0.006)		1032
		72	0.51	12.S	4912
H	4	6	0.60	15.0	1500
		6	0.09	2.2	6S0
		12	0.027	0.7	520
		24	0.039	1.0	1249
		24	(−0.002)		1700
		72	0.756	1S.9	5649

TABLE 9—*Concluded.*

| SUBJECT | DOSE | INTERVAL | AMOUNT OF STRYCHNIN SULPHATE FOUND: | | VOLUME OF URINE |
			In fractions of a milligram	In percentage of dose	
After oral administration					
	mgm.	*hours*			*cc.*
W	4	6	0.56	14.0	450
		6	0.15	3.7	260
		12	0.10	2.5	300
		12	(−0.01)		620
		—	—	—	—
		36	0.81	20.2	1630
H.	4	6	0.37	9.2	400
		6	0.061	1.5	530
		12	0.03	0.8	430
		12	0.045	1.1	780
		—	—	—	—
		36	0.506	12.6	2140
W.	13	24	1.00	7.7	800
		24	0.065	0.5	950
		24	0.017 (?)	0.1 (?)	750
		—	—	—	—
		72	1.082	8.3	2140
H.	15	24	0.36	2.4	1105
		24	0.40	2.7	950
		24	0.035	0.2	980
		24	(−0.01)		1050
		—	—	—	—
		96	0.795	5.3	4085

weighing 2680 grams; indicating the presence of 0.4 mgm. of strychnin sulphate

B. A dose of 0.8 cc. caused increased reflexes in a frog weighing 33.5 grams; 0.5 cc. was ineffective in a frog weighing 33.4 grams; the mean of the doses—0.65 cc.—is accepted as the minimal effective dose; 5 cc. would cause increased reflexes in frogs weighing a total of 276 grams, indicating the presence of 0.04 mgm. of strychnin sulphate.

The calculations indicate the presence of 0.04 mgm. in C. and of 0.03 mgm. in D. E contained less than 0.006 mgm., and probably none.

Discussion

The tabulated results of the experiments show that the rate of elimination of strychnin by the kidney is much greater in the first six hours following its administration than that in the remaining period during which the poison can be detected in the urine, except in one experiment in which only half as much was excreted in that period as in the succeeding eighteen hours. An average of 70 per cent of the total that the kidneys excrete appears in the urine within this period of six hours, and nearly 90 per cent within the first twelve hours, but they only excrete a total of about 11 to 20 per cent of the amounts administered.

Diuresis hastens the elimination of strychnin by the kidneys, but it does not increase the total amount excreted by them after a single intramuscular dose. In the third experiment the kidneys actually excreted less than those of the same subject after a similar dose in the first experiment.

A comparative analysis of the results of the first four experiments is instructive. In the first experiment the liver eliminated about 84 per cent of the poison, and the kidneys about 58 per cent of the remainder within a period of six hours. When diuresis was induced, in the third experiment, the liver eliminated 87 per cent of the total amount injected, and the kidneys excreted about 75 per cent of the remainder within six hours.

The liver of H. eliminated the poison less actively, 80 per cent of the total dose being thus eliminated in both experiments, and in the second experiment the kidneys excreted only about 32 per cent of the balance within six hours. The liver showed no increased capacity for fixing the poison as the result of the measures employed to induce diuresis, but the kidneys then eliminated 78 per cent of the balance within six hours, the figures being in striking agreement in the two subjects.

It was shown in the previous paper of this series that strychnin leaves the blood stream rapidly and that after an interval of forty minutes following its intravenous injection the plasma held only traces in solution. There is little doubt that the liver fixes the greater part of the poison during that period, and it is pos-

sible that the means employed by us to induce diuresis may at times promote the hepatic circulation and the fixation of the poison by the liver, in addition to promoting its excretion by the kidneys. This would leave much less of the alkaloid to be excreted in the later periods. When the liver removes nearly all of the poison within a short time after its entrance into the blood stream, there is relatively little left for the kidneys to excrete, but diuresis enables them to eliminate the smaller total more rapidly than they would otherwise.

This would explain why so much strychnin was excreted by the kidneys during the first six hours in the fourth experiment, and, assuming that all of it is eventually eliminated by the liver and the kidneys, it explains equally why the total excreted by the kidneys in the third experiment is less than that in the corresponding experiment in which diuresis was not induced.

The liver appears to be the principal organ concerned in the protection of the organism against poisoning by ingested strychnin, by virtue of its capacity for removing large amounts from the circulation promptly, since strychnin is capable of causing death within a few seconds after the intravenous injection of a very large dose, and unless elimination nearly keeps pace with absorption into the circulation little protection is afforded. The kidneys, on the other hand, are better adapted for the removal of traces of the poison from the circulating blood.

A total of 0.027 mgm. was excreted in 520 cc. of urine in a period of twelve hours in the 4th experiment. The concentration being equal to 1 part of strychnin sulphate in about 20,000,000 parts of urine, and, obviously, the concentration in the blood was far less at that time, and still less in the succeeding period of 24 hours, during which a total of 0.039 mgm. was excreted.

This suggests that when the poison leaves the blood stream that portion which is not fixed at once in the liver or kidney is stored in some of the tissues which give it up slowly and that it is then present in the blood in such minute amounts that it is incapable of injuring the central nervous system, but that the kidney is capable of removing it almost quantitatively despite

the extraordinary dilution. If this should prove to be the case, it would afford an interesting example of the coördination of two excretory organs, one doing what might be termed the heavy work of elimination by means of its greater bulk, the other doing the more delicate part by reason of its finer mechanism.

The fact that nearly all of the strychnin is eliminated within the first six hours after it enters the circulation is in harmony with the results observed by Hatcher and Eggleston (loc. cit.) who found that cats, dogs, and guinea pigs recover completely from the effects of toxic doses of strychnin sulphate administered several times a day for a week or more.

The percentage of the poison which was eliminated by the kidneys in our experiments does not differ essentially from that observed by Hatcher and Eggleston and by Hatcher and Smith (16), and the results in those experiments in which diuresis was induced confirm the conclusions of Hatcher and Smith, who stated that:

It is possible that diuresis may contribute to the successful therapeutic treatment in those cases where the amount taken is only slightly in excess of the minimal dose that would be fatal in the absence of treatment, or where the absorption of the poison is very slow, but it must play a minor rôle in the treatment of poisoning where very large doses of strychnin have been taken.

The results of the experiments in which strychnin sulphate was taken orally call for brief consideration. The single oral doses were taken in the morning on rising and they were probably absorbed promptly, the elimination being much like that following intramuscular injection. When the larger total doses were taken over longer periods the liver evidently eliminated much larger amounts than in those experiments in which smaller doses were taken.

W. excreted an average of 0.65 mgm. by the kidneys in twenty-four hours in each of the experiments in which single doses of 4 mgm. were taken, and 1 mgm. in twenty-four hours after beginning the administration of a total of 13 mgm.; H. excreted an average of 0.64 mgm. by the kidneys in each of the experi-

ments in which single doses of 4 mgm. were taken, and only 0.76 mgm. in forty-eight hours after beginning the administration of a total of 15 mgm.   .

The development of organs and of special functions, such as protective reflexes designed to meet biologic needs, occurs so frequently, and it so dominates the picture of physiology both in plants and in animals, that it is more than possible that we are too prone to attribute observed phenomena to such biologic developments; nevertheless, we may be permitted to suggest, without dwelling too strongly upon the point, that the greater capacity of the liver in young animals for destroying or eliminating many vegetable poisons may stand in relation with the following fact. The demands of growth and development in the young necessitate the taking of a relatively greater amount of food than adults require and experimenting with a variety of foods in order to determine those best suited for the individual; furthermore, the young have not acquired the skill and strength necessary for selecting only that food which is preferred, and must therefore be prepared to take whatever food offers with the least expenditure of skill.

Obviously this suggestion lacks force when judged solely by conditions obtaining in a highly civilized society of men, but our biologic developments go much further back than civilization, and the principle is not wholly inapplicable to the young of the most refined society.

One may ask how this suggestion agrees with the relatively greater capacity of the kidneys in adults to eliminate the poison over prolonged periods. As a matter of fact the kidneys of the older individuals have no such increased eliminative capacity. Since the liver eliminates about five-sixths of the total poison ingested it follows that it is of far greater importance and that the kidneys are of wholly secondary importance in this respect: If the capacity of the liver for elimination declines relatively, then the kidneys must continue to aid in the excretion until all of the poison is gotten rid of, for, obviously, all of it must be eliminated eventually, else the body would be destroyed in time if absorption continued. If the very old patients men-

tioned by von Rautenfeld did indeed continue to excrete strychnin in the urine six days after the administration was stopped it merely points to an extraordinary delay in its absorption, or an equally remarkable lack of hepatic efficiency in its elimination, and not to any increased renal function, but the kidney function is essential to life, and while life lasts it must retain this capacity for eliminating poisons.

Summary

1. Strychnin sulphate was administered orally and intramuscularly in single doses of 4 mgm. and in repeated oral doses; the urine was collected in periods of six hours, twelve hours and twenty-four hours, and the amounts of strychnin present were estimated by means of the frog tests previously described.

2. The kidneys excrete amounts equal to 20 per cent of that administered at one time, and a much lower percentage of larger doses taken by the mouth over periods of twelve and twenty-eight hours, respectively.

3. The percentage of the strychnin excreted by the kidneys is a measure of the eliminative efficiency of the liver, rather than that of the kidney itself, for the kidney excretes only that which the liver *fails* to excrete.

4. Diuresis hastens the elimination of strychnin by the kidney, but it does not necessarily increase the total amount eliminated in the urine after a single dose injected intramuscularly, and it may, in fact, be attended with the renal elimination of a smaller total than would occur in a similar experiment without diuresis.

5. The liver appears to be the principal protective organ with reference to acute poisoning by strychnin, the kidney to be concerned mainly with the elimination of traces of the poison which reënter the circulation after having been fixed temporarily in those tissues which are incapable of destroying the poison.

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INDEX TO VOLUME XIX